VOLUME 63 • APRIL 1957 • NUMBER 4

# PATHOLOGY

**A Periodical Devoted to General and Experimental Pathology**

**Development of Fetal Pulmonary Arterioles**
*Robert M. O'Neal, Robert C. Ahlvin, Walter C. Bauer, and Wilbur A. Thomas*

**Structural Changes in Pulmonary Arteries in First Year of Life**
*L. Rosen, D. H. Bowden, and I. Uchida*

**A Plea for Grant Support of the Autopsy**
*Alfred A. Angrist*

**Pulmonary Arteriosclerosis Due to Oxygen, Nitrogen, and Argon Embolism**
*P. J. Barnard*

**Multilobulated Spleen in Association with Congenital Heart Disease**
*S. Donald Greenberg*

**Lesions in Kidneys Removed for Unilateral Hematuria in Sickle-Cell Disease**
*F. K. Mostofi, Colin F. Vorder Bruegge, and L. W. Diggs*

**Effects of Colchicine and Serratia Marcescens Polysaccharide on Protoplasmic Viscosity**
*Edwin T. Nishimura and Joseph H. Baum*

**Hibernating Fat and Pheochromocytoma**
*M. M. Melicow*

**Some Observations on the Histochemical Reactions of Amyloid**
*G. M. Windrum and H. Kramer*

**Duplication of Large Intestine Associated with Multiple Malformations**
*Frederick P. Bornstein*

**Experimental Aberrant Lipogenesis**
*David G. Cogan and Toichiro Kuwabara*

**Cross-Circulation and Temporary Arteriovenous-Shunt Lesions in Rabbits**
*Richard L. Davis, Joel G. Brunson, and Peter E. Fehr*

**Acanthosis Nigricans and Carcinoma of the Stomach**
*Bela Halpert and Raymond G. Gottschalk*

**Necrotizing Vascular Disease in Rat**
*Simon Koletsky*

**Allergic Granuloma**
*Henry B. Marsden and John K. Morgan*

**Experimental Xanthomatosis in the Rabbit**
*Chun-I Wang, Lotte Strauss, and David Adlersberg*

**Esterase Activity of Developing Bones and Teeth**
*M. S. Burstone*

**News and Comment**

**Book Reviews**

AMERICAN MEDICAL ASSOCIATION PUBLICATION

# Papanicolaou Stains

standard

for

cytodiagnosis

Ortho Pharmaceutical Corporation

RARITAN, NEW JERSEY

Ortho

# TABLE OF CONTENTS

VOLUME 63 APRIL 1957 NUMBER 4

## ORIGINAL ARTICLES

PAGE

**Development of Fetal Pulmonary Arterioles**
*Robert M. O'Neal, M.D.; Robert C. Ahlvin, M.D.; Walter C. Bauer, M.D., and Wilbur A. Thomas, M.D., St. Louis* ..... 309

**Structural Changes in Pulmonary Arteries in First Year of Life**
*L. Rosen, M.D.; D. H. Bowden, M.B., Ch.B., M.R.C.P., and I. Uchida, Ph.D., Toronto, Canada* ..... 316

**A Plea for Grant Support of the Autopsy**
*Alfred A. Angrist, M.D., New York* ..... 318

**Pulmonary Arteriosclerosis Due to Oxygen, Nitrogen, and Argon Embolism**
*P. J. Barnard, M.D., Pretoria, Union of South Africa* ..... 322

**Multilobulated Spleen in Association with Congenital Heart Disease**
*S. Donald Greenberg, M.D., Houston, Texas* ..... 333

**Lesions in Kidneys Removed for Unilateral Hematuria in Sickle-Cell Disease**
*F. K. Mostofi, M.D., Washington, D. C.; Lieut. Col. Colin F. Vorder Bruegge (MC), U. S. Army, and L. W. Diggs, M.D., Washington, D. C.* ..... 336

**Effects of Colchicine and Serratia Marcescens Polysaccharide on Protoplasmic Viscosity**
*Edwin T. Nishimura, M.D., and Joseph H. Baum, B.S., Chicago* ..... 352

**Hibernating Fat and Pheochromocytoma**
*M. M. Melicow, M.D., New York* ..... 367

**Some Observations on the Histochemical Reactions of Amyloid**
*G. M. Windrum, M.B., D.Phil., and H. Kramer, B.M., D.Phil., Brisbane, Queensland, Australia* ..... 373

**Duplication of Large Intestine Associated with Multiple Malformations**
*Frederick P. Bornstein, M.D., El Paso, Texas* ..... 379

**Experimental Aberrant Lipogenesis**
*David G. Cogan, M.D., and Toichiro Kuwabara, M.D., Boston* ..... 381

**Cross-Circulation and Temporary Arteriovenous-Shunt Lesions in Rabbits**
*Richard L. Davis, M.D.; Joel G. Brunson, M.D., and Peter E. Fehr, B.A., Minneapolis* ..... 387

**Acanthosis Nigricans and Carcinoma of the Stomach**
*Bela Halpert, M.D., and Raymond G. Gottschalk, M.D., Houston, Texas* ..... 400

**Necrotizing Vascular Disease in Rat**
*Simon Koletsky, M.D., Cleveland, with the Technical Assistance of Carol Van Rooy and Annette Press* ..... 405

**Allergic Granuloma**
*Henry B. Marsden, M.B., D.C.H., D. Path., and John K. Morgan, M.D., M.R.C.P., Pendlebury, Lancs., England* ..... 411

**Experimental Xanthomatosis in the Rabbit**
*Chun-I Wang, M.D.; Lotte Strauss, M.D., and David Adlersberg, M.D., New York* ..... 416

## CORRESPONDENCE

**Esterase Activity of Developing Bones and Teeth**
*M. S. Burstone, Bethesda, Md.* ..... 426

## REGULAR DEPARTMENTS

**News and Comment** ..... 422

**Book Reviews** ..... 425

# A. M. A.
# ARCHIVES of PATHOLOGY

**Also the Official Organ of the AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY**

VOLUME 63 APRIL 1957 NUMBER 4

COPYRIGHT, 1957, BY THE AMERICAN MEDICAL ASSOCIATION

EDITORIAL BOARD

PAUL R. CANNON, Chief Editor
Department of Pathology, University of Chicago,
The School of Medicine, 950 E. 59th St., Chicago 37

GRANVILLE A. BENNETT, Chicago
CHARLES E. DUNLAP, New Orleans
WILEY DAVIS FORBUS, Durham, N. C.
STUART LIPPINCOTT, Upton, L. I., N. Y.
SIDNEY C. MADDEN, Los Angeles
WILLIAM MEISSNER, Boston
WILLIAM B. WARTMAN, Chicago
GEORGE H. WHIPPLE, Rochester, N. Y.

AUSTIN SMITH, Editor, A. M. A. Scientific Publications
GILBERT S. COOPER, Managing Editor, Specialty Journals

SUBSCRIPTION RATES

Price per annum in advance, including postage: Domestic, $10.00. Canadian, $10.50. Foreign, $11.50. Price to students, interns, and residents, $6.00 in U. S. & possessions.

Single copies of this and previous calendar year, $1.00 each. Back issues older than two years are available through Walter J. Johnson, Inc., 111 Fifth Avenue, New York 3. Future reprints of back issues will be available through Johnson Reprint Corporation, 111 Fifth Avenue, New York 3.

Checks, money orders, and drafts should be made payable to the American Medical Association, 535 North Dearborn Street, Chicago 10.

**AMERICAN MEDICAL ASSOCIATION Publication**

Published monthly by the AMERICAN MEDICAL ASSOCIATION. Editorial and Circulation Offices: 535 North Dearborn Street, Chicago 10, Illinois. Publication Office: Thompson Lane, Box 539, Nashville 1, Tennessee. Change of Address: Notice to the circulation office should state whether or not change is permanent and should include the old address. Six weeks' notice is required to effect a change of address. Second-class mail privileges authorized at Nashville, Tenn., Aug. 6, 1936.

# Paragon Tray Drawer Cabinet

*Compact* *Low Cost*

**FOR FILING**
**MICROSCOPIC SLIDES 3 x 1"**
**KODACHROME TRANSPARENCIES 2 x 2" SLIDES**
**LANTERN SLIDES (up to 3¼ x 4¼)**
**PETROGRAPHIC SLIDES**

*When you purchase a*
**PARAGON TRAY DRAWER CABINET**
**YOU PURCHASE FILING SPACE ONLY**
**NO WASTE SPACE-EVERY INCH USED**

U. S. Pat. No. 2,202,047
C101—Tray Drawer Cabinet for 3 x 1 Micro Slides
Capacity 4500—18¾ x 15¾ x 4¼

All Paragon Tray Drawer Cabinets are manufactured in standard sizes so that any number of sections may be interlocked to form one cabinet to accommodate any number of varied slides. The dimensions of the different cabinets are the same as to length and width, varying only in height. The cabinet formed by interlocking may be 18¾ x 15¾; 18¾ x 11 or 18¾ x 5 or it may be a pyramid with the sections varying in width.

C221—Capacity 1500 Slides—18¾ x 11 x 3¾
**For Filing KODACHROME TRANSPARENCIES and 2 x 2" SLIDES**

**SPECIFICATIONS:** All Paragon Tray Drawer Cabinets are made of reinforced steel construction, olive green finish. Interlocking device enables several units to be joined into one. Each sectional unit contains removable drawers with hand grip in front and rear. Interlocking steel base obtainable whenever required. **Constructed according to rigid specifications—not merely adapted.**

**Address your orders and inquiries to Dept. P.**

*Manufactured Exclusively by*

**PARAGON C. & C. CO., Inc. • 2540 Belmont Ave., New York 58, N.Y.**

## Instructions to Contributors

Articles, book reviews, and other materials for publication should be addressed to the Chief Editor of the Specialty Journal concerned. Articles are accepted for publication on condition that they are contributed solely to that journal.

An original typescript of an article, with one carbon copy, should be provided; it must be double or triple spaced on one side of a standard size page, with at least a 1-inch margin at each edge. Another carbon copy should be retained by the author. An article in English by a foreign author should be accompanied by a draft in the author's mother tongue. Improvised abbreviations should be avoided.

The main title of an article may not contain more than eighty characters and spaces; a subtitle may be of any length. The subtitle should be sufficiently detached from the main title that a punctuation mark as strong as a colon can be used to separate it from the main title; it must not begin with "with."

The author's name should be accompanied by the highest earned academic or medical degree which he holds. If academic connections are given for one author of an article, such connections must be given for all other authors of the article who have such connections.

If it is necessary to publish a recognizable photograph of a person, the author should notify the publisher that permission to publish has been obtained from the subject himself if an adult, or from the parents or guardian if a child. An illustration that has been published in another publication should be accompanied by a statement that permission for reproduction has been obtained from the author and the original publisher.

Oversized original illustrations should be photographed and a print on glossy paper submitted. Prints of a bluish tinge should be avoided. Large photomicrograph prints will be reduced in scale unless portions to be cropped are indicated by the author. The author should submit duplicate prints of roentgenograms and photomicrographs with the essential parts that are to be emphasized circled, as a guide to the photoengraver.

Charts and drawings should be in black ink on hard, white paper. Lettering should be large enough, uniform, and sharp enough to permit necessary reduction. Glossy prints of x-rays are requested. Paper clips should not be used on prints, since their mark shows in reproduction, as does writing on the back of prints with hard lead pencil or stiff pen. Labels should be prepared and pasted to the back of each illustration showing its number, the author's name, and an abbreviated title of the article, and plainly indicating the top. Charts and illustrations must have descriptive legends, grouped on a separate sheet. Tables must have captions. ILLUSTRATIONS SHOULD BE UNMOUNTED.

References to the literature should be numbered in the order in which they are referred to in the text or listed in alphabetical order without numbers. A chronological arrangement, with all entries for a given year alphabetized according to the surname of the first author, may be used if preferred. References should be typed on a special page at end of manuscript. They should conform to the style of the Quarterly Cumulative Index Medicus, and must include, in the order given, name of author, title of article (with subtitle), name of periodical, with volume, page, month—day of month if weekly or biweekly—and year. Names of periodicals should be given in full or abbreviated exactly as in the Quarterly Cumulative Index Medicus. Reference to books must contain, in the order given, name of author, title of book, city of publication, name of publisher, and year of publication. Titles of foreign articles, if originally in a generally known Romance or Germanic tongue, must either all be in English translation or all be in the original language. Titles in other languages must be translated. The author must assume responsibility for the accuracy of foreign titles.

**AMERICAN MEDICAL ASSOCIATION**

**535 North Dearborn Street** **Chicago 10**

# International's new high-speed angle centrifuge model HT

**A fully enclosed table model with speeds up to 17,000 r.p.m.*, forces up to 34,390 G* and capacity of 400 ml.**

*featuring*

**Complete Protection for the Operator . . .**
Two thicknesses of heavy-gauge steel — guard bowl *plus* cabinet — protect operator from rotating head. Cover of thick plate steel is held securely with rugged hinge and lever lock.

**Removable Instrument Panel for Remote Control . . .**
Front panel unit, containing all instruments and controls can be removed easily from cabinet and is portable, allowing remote control operation when centrifuge is located in a cold room or chamber.

**Complete Instrumentation . . .**
**Electric Tachometer** constantly indicates true operating speed, regardless of line voltage fluctuations.

**Ammeter** shows current flow for acceleration control.

**Automatic Timer** shuts off centrifuge at selected time interval. Timer can be eliminated by cutout switch.

**Autotransformer** gives stepless speed control without heating.

Protective No-Voltage Relay, Pilot Light and Safety Fuse.

**Unique Cooling System . . .**
A specially designed air circulation system provides maximum cooling of head and bearings.

*Note: speeds are obtained at 115 volts input to centrifuge; force is effective centrifugal force figured at the maximum radius of the material being centrifuged.

Designed for Safe Operation

Centrifuge Inside — Controls Outside the Cold Room or Chamber

**WRITE FOR BULLETIN E**

**International Equipment Co.**

1284 SOLDIERS FIELD ROAD • BOSTON 35, MASSACHUSETTS

## BRING YOUR PATIENT'S HIDDEN INFLAMMATION TO LIGHT WITH

# C·R·P·A®

*(C-reactive Protein Antiserum, Schieffelin)*

- **C-Reactive protein in serum only when inflammation exists.**
- **Unlike E.S.R., no "Normal" range...either negative or positive.**
- **No false positives.**
- **Simpler . . . faster . . . more reliable aid to diagnosis.**
- **Quantity of precipitate reflects severity of condition . . . Precipitate diminishes as therapy reduces inflammation.**

Both venous blood and blood obtained by the "finger tip technique" (punctured as for blood count) can be used with equally reliable results. To make the test procedure fast and convenient a new compact C.R.P.A. Test Kit is now available; it contains C.R.P.A., CRP-Positest control solution, capillary tubes, test rack, and illustrated directions.

**COMPLETE LITERATURE ON C.R.P.A. IN DETECTION OF RHEUMATIC DISEASES, MYOCARDIAL INFARCTION, AND MANY OTHER DISEASE STATES ON REQUEST.**

Supplied:

TEST KIT *(For the complete procedure)*

C.R.P.A. . . . 1 ml. vials (80-110 determinations)

CRP-POSITEST* . . . 0.5 ml. vials
*(Standardized test-control solution of C-Reactive Protein)*

CAPILLARY TUBE RACK

CAPILLARY TUBES
(Two sizes) . . . 0.8 mm ID – 1.5 mm ID

*Trademark

Schieffelin & Co.

New York 3, N. Y. • Since 1794

Laboratory Products Division

*In Canada: William Sofin & Co., Ltd., Montreal 25, Quebec*

A.M.A. ARCHIVES OF
PATHOLOGY

# Development of Fetal Pulmonary Arterioles

*Morphologic Study of Normal Development of the Muscular Coat in Fetal Pulmonary Arterioles During the Latter Half of Intrauterine Life*

ROBERT M. O'NEAL, M.D.; ROBERT C. AHLVIN, M.D.; WALTER C. BAUER, M.D., and WILBUR A. THOMAS, M.D., St. Louis

A common cause of neonatal death associated with prematurity is respiratory failure ("hyaline membrane disease"). The intense pulmonary capillary congestion and exudation of fibrin and red blood cells that characterize "hyaline membrane disease" pathologically [1] and the recent demonstration that "hyaline membranes" are derived in part from plasma proteins [2] suggest that this condition may be the result of a pulmonary circulatory disturbance. Knowledge of the development of the pulmonary vasculature, particularly the terminal segments of the pulmonary arterial tree, may lead to the recognition of important anatomic factors which predispose premature infants to pulmonary circulatory disturbances resulting in respiratory distress and "hyaline membrane."

Studies of the structure of pulmonary arterioles in infants have been reported only rarely, and the time and manner of development of arteriolar media in fetal life have not been described. The purpose of this report is to present the results of a morphologic study of the pulmonary arterioles and the changes that occur in them during the latter part of intrauterine life. Such a study should establish a standard for the evaluation of the amount of arteriolar media in individual cases and provide a basis for the morphologic determination of the state of prematurity of the pulmonary vasculature.

Submitted for publication Aug. 20, 1956.

From the Department of Pathology, Washington University School of Medicine.

This investigation was supported in part by a research grant H-2359 from the National Heart Institute, National Institutes of Health, U. S. Public Health Service.

## Materials and Methods

The anatomic material was obtained from the files of the department of pathology of Washington University. Sixty-two stillborn infants and newborn infants less than one week of age at the time of their death were studied. Those with cardiovascular anomalies or hydrops fetalis were excluded. By the use of a graph prepared from Streeter's data of fetal ages and weights [3] (Fig. 1), the cases were divided into age groups on the basis of their birth weights. Accordingly, a birth weight of over 2900 gm. was considered in this study to represent a fetal age of at least 9.5 lunar months and was presumed to be full term. The fetal ages of eight of the infants were over 9.5 lunar months. Nine were between 9.5 and 9 months; eight were between 9 and 8.5 months; nine were between 8.5 and 8 months; eight were between 8 and 7.5 months; seven were between 7.5 and 7 months; eight were between 7 and 6.5 months; three were between 6.5 and 6 months, and two were between 6 and 5.5 months.

Paraffin blocks of Zenker-formol-fixed lung that had been taken at the time of autopsy were recut and stained with aldehyde fuchsin-Van Gieson-iron hematoxylin. This combination of stains differentiates fibrous tissue, elastic tissue, and muscle

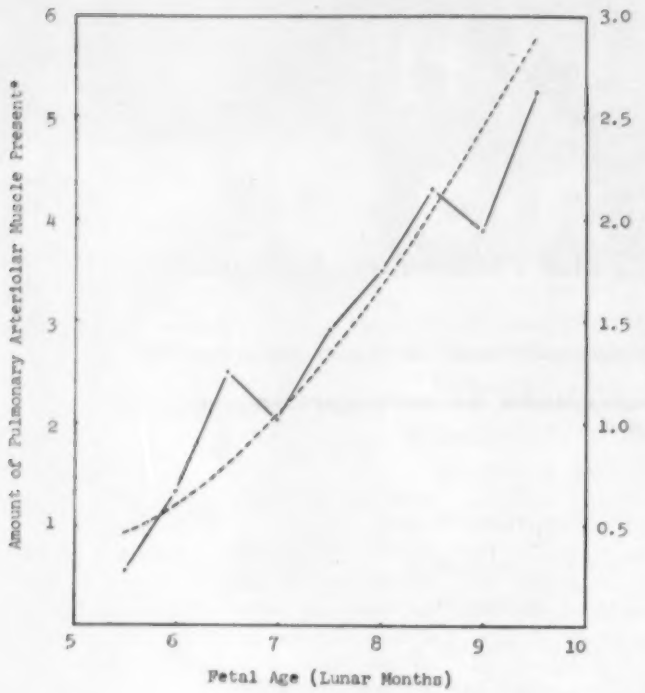



Fig. 1.—Relationship of the amount of pulmonary arteriolar muscle to increasing fetal age. Solid line connects points representing the average amount of pulmonary arteriolar muscle in each age group. Note the gradual increase in muscle as the fetal age approaches term. The dotted line shows the fetal weight-fetal age relationship as determined by Streeter.[3] Fetal age groups were derived from these data.

* The grading system from which these units were derived is described in the text.

as satisfactorily as the Verhoeff-Van Gieson method and is technically easier to perform. We have found that overdifferentiation of the Verhoeff stain is a constant hazard, especially in the lungs of infants. Staining was performed as described by Lillie[4] except for minor modifications.

The tissue sections were studied by two of us independently. Arteriolar muscle was graded according to a modification of a system previously described by us.[5] The relative width of the arteriolar muscle ("thickness") was graded 0, 1, 2, or 3, the highest grade indicating medial thickness equal to that present in systemic arterioles. The frequency of occurrence of muscular arterioles ("extent") was also graded 0, 1, 2, or 3, the highest grade indicating that all recognizable arterioles had muscular walls.

In a particular case the grade of "thickness" assigned was that of the most heavily muscled arteriole identified, while the grade of "extent" was arrived at by assessing the frequency of arterioles which had muscular walls. These two numbers, the grade of the "thickness" of the arteriolar walls and that of the "extent" of the muscular arterioles, were added in each case. The arithmetical mean of these combined grades was calculated for each age group. The resulting figure served as an arbitrary index of the amount of pulmonary arteriolar muscle present in each age group.

## Results

The differentiation of small arteries and arterioles in the lungs of newborn infants is difficult, and therefore we devised the following specific definitions used in this study. Small muscular arteries lie adjacent to peripheral branches of the bronchial tree which are lined by recognizable respiratory epithelium. These arteries extend into the pulmonary parenchyma to the end of the bronchioles. We define a vessel as an arteriole (1) if it is seen to originate from a small muscular artery that lies adjacent to a terminal ("respiratory") bronchiole (Figs. 2, 3, 4, and 5) or (2) if it lies in the wall of a terminal air space within a pulmonary lobule but away from a bronchiole (Fig. 6). In the latter instance, if the vessel does not have a recognizable media, it cannot be differentiated from a venule.

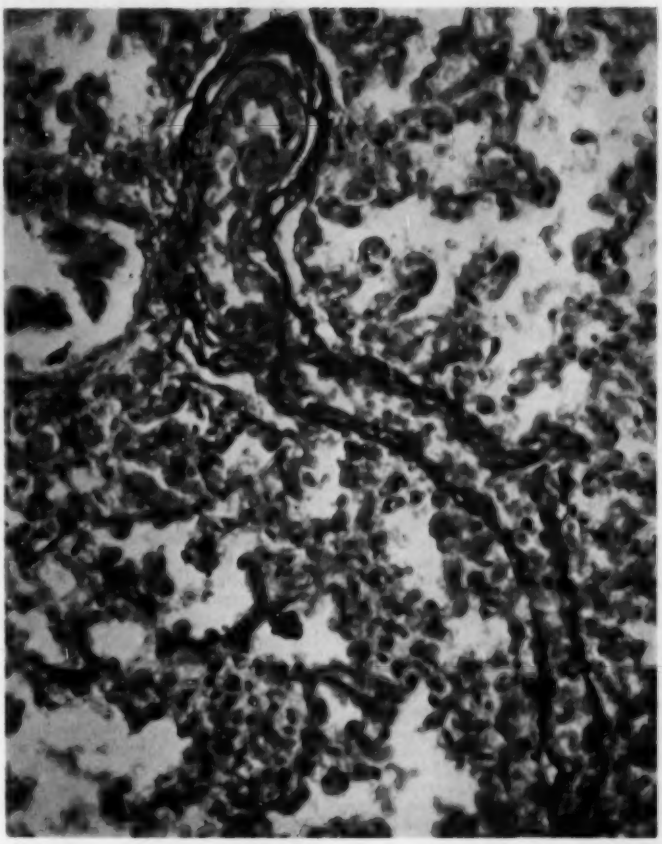


Fig. 2.—Lung of a newborn infant of 9 lunar months' gestation. At the upper left is a small muscular artery lying beside a respiratory bronchiole. The lumen of the bronchiole contains sloughed epithelial cells. An arteriole arises from the small muscular artery and extends to the lower right. Smooth muscle lies between the internal and external elastic laminae in the proximal half of the arteriole, but in the distal half the arteriolar wall is composed only of endothelial cells overlying a single elastica. Aldehyde fuchsin - Van Gieson-iron hematoxylin; reduced to 80% of mag. × 680.

Using these criteria, pulmonary arterioles could be readily identified in the lungs of the infants used in this study. The aldehyde fuchsin-Van Gieson-iron hematoxylin technique stained the elastic fibers dark blue and the cytoplasm of smooth muscle cells light yellow. When arteriolar media was present, the external and internal elastic laminae could usually be identified with ease and the smooth muscle cells could be seen between them. In the full-term infants, the external diameter of an arteriole near its origin was approximately 25$\mu$. A well-defined media, continuous with the media of the small muscular artery from which it arises, was present, and the wall of the vessel was approximately as thick as the diameter of its lumen.

In longitudinally cut arterioles, it was often possible to demonstrate the two elastic laminae enclosing smooth muscle only in the proximal portion of the arteriole; in the distal portion the arteriolar wall was composed of a single elastic lamina and overlying endothelium (Figs. 2, 4). The length of arteriolar wall that contained media appeared to increase with the increasing maturity of the fetus. The elastic laminae of an arteriole were continuous with those of the small muscular artery from which the arteriole arose. A continuous external elastic lamina in the arteriolar walls was easily identified even in the youngest fetuses studied, but the internal elastica was often delicate or discontinuous in the most immature fetuses. The internal elastic lamina became more prom-

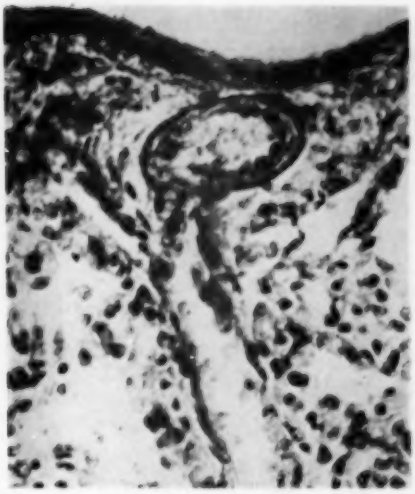


Fig. 3.—Lung of a newborn infant of 7.5 lunar months' gestation. The small muscular artery lying near the wall of a respiratory bronchiole has a distinct external elastic lamina, well-defined smooth muscle, and a faint internal elastic lamina. The pulmonary arteriole arising from this artery has a very thin wall without any smooth muscle apparent. Aldehyde fuchsin-Van Gieson-iron hematoxylin; × 330.

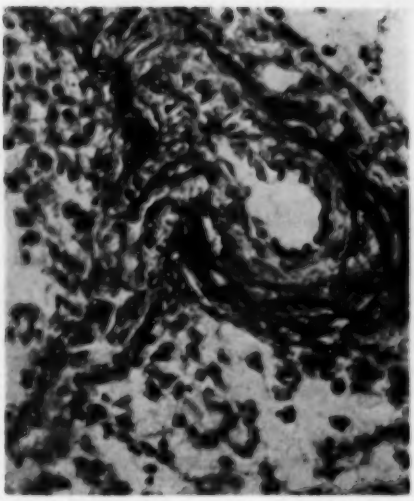


Fig. 5.—Lung of a newborn infant of 9 lunar months' gestation. The arteriole branching from this small muscular artery has a thick, muscular wall. The muscle coat in the small muscular artery appears thicker than that in Figure 1, but this could be due to a difference in the degrees of contraction of the two vessels. Aldehyde fuchsin-Van Gieson-iron hematoxylin; × 357.

Fig. 4.—Lung of a newborn infant of 8 lunar months' gestation. At the right a small muscular artery is seen following the wall of a respiratory bronchiole. The vessel branches into arterioles just beyond the point at which the cuboidal bronchial epithelium ends. The most proximal portion of the arteriole has smooth muscle between the endothelium and the external elastica. In the more distal portion of the arteriole (upper left) the endothelial cells lie directly on the single elastic lamina, demonstrating the absence of smooth muscle in the arteriolar wall at this point. Aldehyde fuchsin-Van Gieson-iron hematoxylin; × 400.

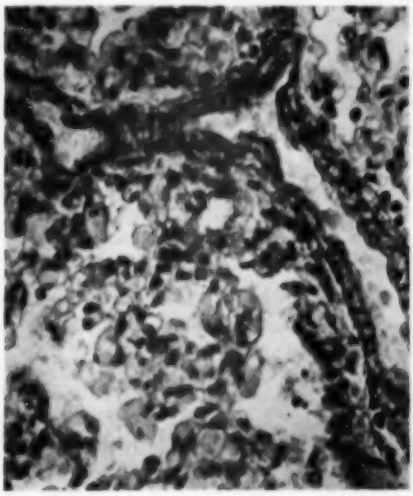


Fig. 6.—Lung of a newborn infant of 9 lunar months' gestation. Cross section of an arteriole. This vessel lies well away from a bronchiole; it is surrounded by peripheral air spaces. There is definite smooth muscle between the delicate internal elastica and the heavy external elastica. The four nuclei of endothelial cells overlie the internal elastica. The lumen is very narrow. Muscular arterioles, such as this one, are not seen in normal infants over 6 months of age (postnatal) or in very premature newborn infants. Aldehyde fuchsin-Van Gieson-iron hematoxylin; × 1200.

*Statistical Analysis of Data for Four Fetal Age Groups*

| | Fetal Age Groups (Lunar Mo.) | | | |
|---|---|---|---|---|
| | <7 | 7-8 | 8-9 | >9 |
| No. of fetuses | 13 | 15 | 17 | 17 |
| Arithmetical mean* | 1.06 | 2.47 | 3.94 | 4.86 |
| Standard deviation of mean | 1.91 | 1.74 | 1.73 | 1.31 |
| *t* value (comparing group <7 mo. with group >9 mo.) Significant at 1% level | -- | -- | 4.03 | |
| *t* value (comparing group 7-8 mo. with group 8-9 mo.) Significant at 5% level | -- | -- | 2.33 | |

* The grading system from which these means were derived is described in the text.

inent with the increasing maturity of the fetus, and its full development appeared to lag slightly behind the appearance of smooth muscle in the arteriolar wall.

The results of the grading of arteriolar muscle are plotted in Figure 1. The arithmetical mean of the combined grades of "thickness" and "extent" was 5.25 in full-term infants, 3.9 in infants of 9.5 to 9 lunar months' gestation, 4.3 in infants of 9 to 8.5 months' gestation, 3.5 in infants of 8.5 to 8 months' gestation, 2.9 in infants of 8 to 7.5 months' gestation, 2.0 in infants of 7.5 to 7 months' gestation, 2.5 in infants of 7 to 6.5 months' gestation, 1.33 in infants of 6.5 to 6 months' gestation, and 0.5 in infants of 6 to 5.5 months' gestation.

The data were subjected to statistical analysis by calculating the significance of the differences in arithmetical means[6] of each of four groups: those fetuses of less than 7 lunar months' gestation; those of 7 to 8 months' gestation; those of 8 to 9 months' gestation, and those of more than 9 months' gestation (Table). The difference between the mean of the group of fetuses of less than 7 lunar months' gestation and the mean of that group of over 9 lunar months' gestation was highly significant (*t* value 4.06; *P* less than 1%). When the means of the two middle groups were compared, they similarly were found to be significantly different (*t* value 2.33; *P* less than 5%). The arithmetical mean and standard deviation for each of the four groups are shown in Figure 7.

It should be pointed out that the two factors, "thickness" and "extent," taken separately also increase with the increasing age of the fetus, and when either factor is used alone in the statistical analysis of the difference of the means of the youngest and oldest fetal age groups, the resulting *t* value is also significant ("thickness," *t* value 2.47, *P* 2%; "extent," *t* value 4.66, *P* less than 1%).

## Comment

Studies of the structure of pulmonary arterioles in infants have been reported only rarely. A deterrent to the study of these vessels in infants has been the lack of a widely accepted and recognized definition of the term "arteriole" as applied to the pulmonary vasculature. Therefore it seems necessary to define anatomically the pulmonary arteriole. The studies of Brenner[7] and Edwards[8] have provided the basis for our definition. In general terms, pulmonary arterioles are those vessels in the pulmonary arterial tree that link small muscular pulmonary arteries, which lie along peripheral bronchioles, to capillaries. Therefore any precapillary branch of the pulmonary arterial tree that lies in the wall of an air space, away from a bronchiole, is an arteriole.

In adults, pulmonary arterioles are characterized by the absence of a continuous muscular coat in their walls[7,8] and therefore can be distinguished with certainty from venules only by observing their origins from small muscular arteries in a fortuitously cut section or in serial sections. They are much larger than the "metarterioles" of the systemic circulation described by Zweifach.[9]

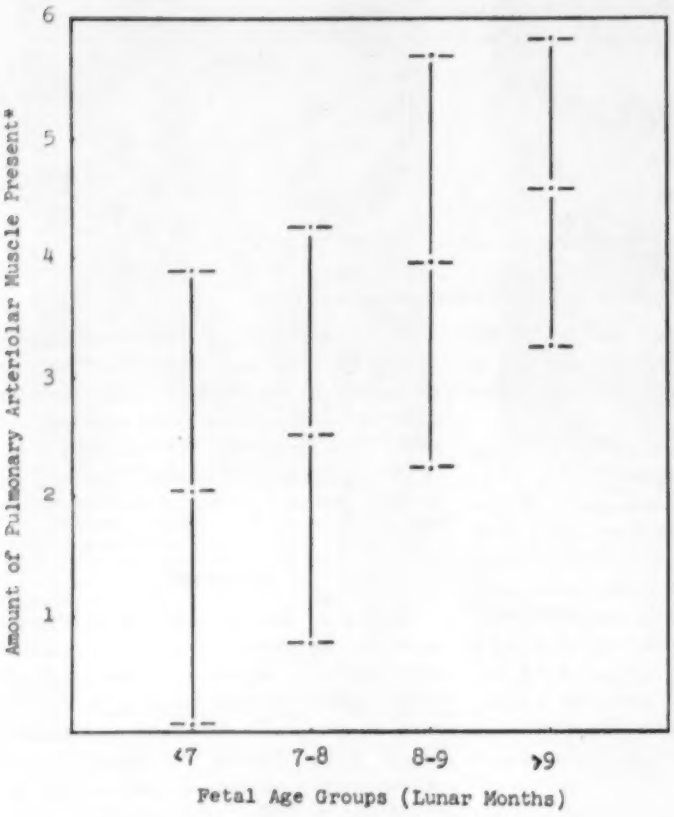



Fig. 7. — Pulmonary arteriolar muscle and fetal age. The middle dot represents the arithmetical mean of the amount of pulmonary arteriolar muscle present in each fetal age group. The upper and lower dots represent one standard deviation from the mean. The difference between the mean of the group <7 and the mean of the group >9 is highly significant ($t$ value 4.06; $P$ less than 1%). The difference between the means of the two middle groups is also significant ($t$ value 2.33; $P$ less than 5%).

* The grading system from which these units were derived is described in the text.

In newborn full-term infants, the pulmonary arterioles possess a thick muscular media similar to that of systemic arterioles [10] and therefore can be readily distinguished from venules. They cannot, however, be distinguished from small muscular arteries (adjacent to bronchioles) except by their position in the walls of the peripheral air spaces. The thick arteriolar media is probably important in maintaining a high intrapulmonary resistance to blood flow during the latter part of intrauterine life and thereby facilitating flow through the ductus arteriosus from the pulmonary artery to the aorta. The arteriolar media may also be important in preventing too rapid an increase in pulmonary blood flow in the immediate postnatal period. However, resistance to pulmonary blood flow in the fetus and newborn is not the result of arteriolar resistance alone. Civin and Edwards [11] have pointed out that the small muscular arteries of the fetus also have very thick mediae and narrow lumina.

Normally the pulmonary arteriolar muscle disappears within a few months after birth,[10] although the exact time of disappearance of the muscular coat has not been definitely established, and we are not primarily concerned with this problem at the present time. In a previous study, no pulmonary arteriolar muscle could be demonstrated in infants over 6 months of age with normal cardiovascular systems.[10] In another study, we (R. M. O.'N. and W. A. T.) demonstrated that abnormal persistence of muscular arterioles of the type seen in normal full-term newborn infants was com-

mon in young children with congenital cardiovascular anomalies that permitted a shunt of blood from the left to the right side of the heart.[5] These children presumably had had pulmonary hypertension since birth.

The results of this study indicate that smooth muscle develops gradually in the walls of fetal pulmonary arterioles during the latter half of pregnancy. The smooth muscle appears first in the most proximal portion of the arteriole and with increasing maturity extends peripherally. A delicate and incomplete internal elastic lamina overlies the smooth muscle in the pulmonary arterioles of premature fetuses, but near the end of the intrauterine life this inner elastica is usually complete, though still more delicate than the outer elastica. The external elastic lamina is present in the arterioles of even the youngest fetuses examined in this study.

The paucity of arteriolar media in premature infants may well be an important factor predisposing these children to pulmonary disturbances resulting in respiratory distress, and the pathologic condition commonly referred to as "hyaline membrane disease."

## Summary

The lungs of 62 stillborn or newborn infants were examined to determine the amount of smooth muscle in the walls of pulmonary arterioles. The amount of smooth muscle in arteriolar walls increases gradually during the latter half of intrauterine life. The relative lack of arteriolar muscle in premature infants may predispose these children to pulmonary circulatory disturbances resulting in respiratory failure.

Department of Pathology, Washington University School of Medicine, Euclid Ave. & Kingshighway (Dr. Thomas).

## REFERENCES

1. Potter, E. L.: Pathology of the Fetus and the Newborn, The Year Book Publishers, Inc., Chicago, 1953.

2. Gitlin, D., and Craig, J. M.: The Nature of the Hyaline Membrane in Asphyxia of the Newborn, Pediatrics 17:64-71, 1956.

3. Streeter, G. L.: Weight, Sitting Height, Head Size, Foot Length, and Menstrual Age of the Human Embryo, Contrib. Embryol. 11:143-170, 1920.

4. Lillie, R. D.: Histopathologic Technique and Practical Histochemistry, New York, The Blakiston Company, 1954.

5. O'Neal, R. M., and Thomas, W. A.: The Role of Pulmonary Hypertension and Thromboembolism in the Production of Pulmonary Arteriosclerosis, Circulation 12:370-382, 1955.

6. Snedecor, G. W.: Statistical Methods, Ed. 4, Ames, Iowa, Iowa State College Press, 1955.

7. Brenner, O.: Pathology of the Vessels of the Pulmonary Circulation, Arch. Int. Med. 56: 212-237, 1935.

8. Edwards, J. E.: Structural Changes of the Pulmonary Vascular Bed and Their Functional Significance in Congenital Cardiac Disease: 26th Ludwig Hektoen Lecture, Proc. Inst. Med. Chicago 18:134-146, 1950.

9. Zweifach, B. W.: Basic Mechanisms in Peripheral Vascular Homeostasis, in Factors Regulating Blood Pressure, edited by B. W. Zweifach and E. Shorr, Transactions of Third Conference, May 5-8, 1949, New York, Josiah Macy, Jr. Foundation, 1949, p. 13.

10. Valenzuela, C. T.; Toriello, J., and Thomas, W. A.: Structural Changes in Intrapulmonary Arteries Exposed to Systemic Pressures from Birth, A. M. A. Arch. Path. 57:51-61, 1954.

11. Civin, W. H., and Edwards, J. E.: Postnatal Structural Changes in the Intrapulmonary Arteries and Arterioles, A. M. A. Arch. Path. 51:192-200, 1951.

# Structural Changes in Pulmonary Arteries in First Year of Life

L. ROSEN, M.D.; D. H. BOWDEN, M.B., Ch.B., M.R.C.P., and I. UCHIDA, Ph.D., Toronto, Canada

Physiological and pathological variations of pulmonary arterial pressure and flow are reflected in structural changes in the small pulmonary arteries and arterioles. In fetal life and shortly after birth, these vessels resemble the systemic arterioles, the lumen is small, and the media is thick (Fig. 1*A*). During the early months of life, particularly in the first two to four weeks, these vessels undergo a change in structure, whereby the media thins as the lumen diameter increases (Fig. 1*B*). We wish to present a simple method of measuring these changes and the figures obtained in normal infants from birth to one year of age.

Submitted for publication Sept. 26, 1956.

Mr. Bowden's present address: Department of Pathology, Cardinal Glennon Memorial Hospital for Children, St. Louis.

## Methods

Sections of lungs from 59 infants under one year of age were examined. None of the cases showed clinical or pathological evidence of pulmonary hypertension, and there were no cardiac anomalies in the group. Sections of the lungs,

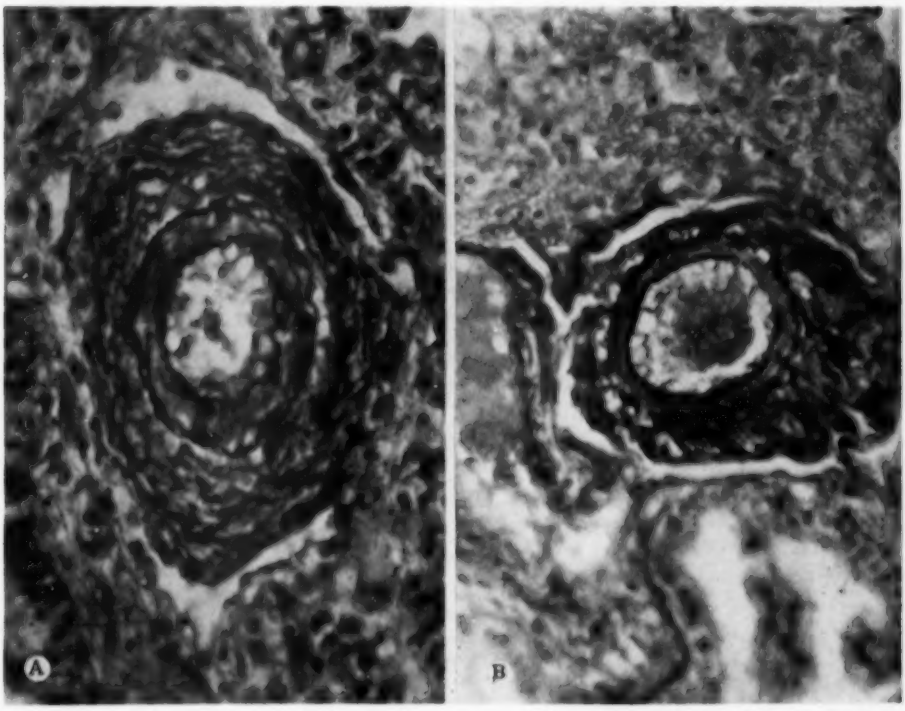



Fig. 1.—*A*, small pulmonary artery from normal newborn infant (ratio 0.59). *B*, small pulmonary artery from normal 7-month-old infant (ratio 0.77). Verhoeff's elastic stain.

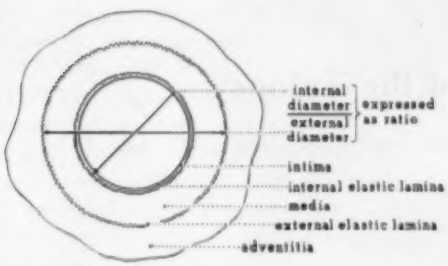



Fig. 2.—Method of measuring diameters of artery.

fixed in Zenker's solution, were stained with Verhoeff's elastic stain.

The method described by O'Neal, Thomas, and Hartroft[1] was used, in which two classes of small muscular arteries were measured: those lying adjacent to the origin of a respiratory bronchiole, and those vessels lying adjacent to a peripheral respiratory bronchiole. Only vessels cut completely or approximately at right angles to their long axis were measured. An ocular scale was used, and two measurements were made on each vessel (Fig. 2).

A total of 652 observations were made on the 59 cases. O'Neal, Thomas, and Hartroft expressed their results as the area of the media. We have used a single expression, the ratio of the internal diameter (lumen and intima) to the external diameter (lumen, intima, and media), i.e., internal diameter/external diameter. The resultant fraction is a measure of the relative thickness of the media as compared with the size of the lumen, the figure being inversely proportional to the area of the media. The ratio, which is similar to that described for systemic arteries by Kernohan, Anderson, and Keith,[2] appears to be a practical measurement, as it allows for ready comparison between cases.

In the two classes of arteries measured the ratios were not significantly different, and therefore the measurements have been combined.

Several measurements were made from each specimen, and a mean ratio was obtained for each of the 59 infants. This sample was divided into two groups according to age: 24 in the group less than one month and 35 one month and more. In the former there were 303 observations, with an average of 12.6 readings per case; in the latter there were 349 observations, with an average of 10.0 readings per case. In the Table are given the means and standard errors for the two age groups. The $t$ value obtained indicates a highly significant difference between these two means ($P<0.001$).

*Mean Ratios of the Two Groups Analyzed*

| Groups | No. of Infants | Mean Ratio, ± Standard Error | |
|---|---|---|---|
| Less than one month | 24 | 0.58±0.016 | $t=6.8$ |
| One month and over | 35 | 0.73±0.015 | |

## Comment

The figures shown in the Table indicate that there is a significant difference between the ratios of the internal diameter to the external diameter in the age groups which were studied. The results confirm the impression that with the establishment of the postnatal pulmonary circulation the small pulmonary arteries dilate and the media becomes thinned. We consider that the ratio offers a simple and reliable method of recording the state of the pulmonary arteries. Correlation of postmortem measurements with pulmonary artery pressures measured during life is, of course, necessary, and we hope to be able to do this in the near future.

## Summary

Variations of pulmonary arterial pressure are reflected in structural changes in the small pulmonary arteries. A simple method is described for the recording of the measurements of these arteries. The figures are expressed in the form of a ratio which facilitates the comparison of different cases.

The normal values for 24 infants under one month of age are given and compared with the figures for 35 children between one month and one year. A significant difference is noted.

Hospital for Sick Children, 555 University Ave. (2).

## REFERENCES

1. O'Neal, R. N.; Thomas, W. A., and Hartroft, P. M.: The Media of Small Muscular Pulmonary Arteries in Mitral Stenosis, A. M. A. Arch. Path. 60:267, 1955.

2. Kernohan, J. W.; Anderson, E. W., and Keith, N. M.: The Arterioles in Cases of Hypertension, Arch. Int. Med. 44:395, 1929.

# A Plea for Grant Support of the Autopsy

*Devaluation of the Autopsy by Present Grant Policy and the Problem It Presents in Medical Education*

**ALFRED A. ANGRIST, M.D., New York**

In a recent editorial, Starr [1] in fact proposes the liquidation of the autopsy and has initiated a sharp controversy. Several outstanding pathologists have responded in defense of the postmortem procedure.[2-6] Cannon's [7] refutation, "Clinical Lessons Learned at the Morgue," notes that "the dominant role of the autopsy in modern medical practice" and points out that "the need for the morgue is steadily mounting, with respect to both the formulation of guiding principles of modern therapy and the development of ideas to be carried to the laboratory." He notes "the fact that the autopsy . . . is assuming research proportions, thereby demanding in its performance greater skills and increasing expenditure of time and money." It may be stated without apologies that good old-fashioned morphology is still not unfruitful. Experimental pathology itself "depends largely upon a sound basis of morphology" (Cowan).[8] Cannon lists 30 new disease entities discovered in recent years by the autopsy. Klemperer [9] has added others, as has Karsner [10]; both have stressed the importance of the autopsy in investigation and education.

Of greater significance than the deemphasis of the autopsy as an instrument of teaching and research is the crystallization by Starr of a more important issue, i. e., a growing loss of perspective in medical education, brought on by the increasing and exclusive emphasis on "pure" research. The increasing stress on instrumentation and the equating of experimental with fundamental research in medical schools are tending to isolate laboratory research and set it up in competition with the clinical and the fundamental teaching functions. This that has led to an unfortunate denial in some departments of pathology of the proper role of the autopsy in medical education.

Research needs no defense, and every effort to promote it is salutary. Its contribution to the modern medical scene has recently been portrayed vividly.[11] Without it, all medical education would be backward, static, and sterile, for student and teacher alike. Yet all medical education cannot be achieved wholly by means of the research technique and procedure. In his "Annual Discourse: *Docendo Discimus*" before the Massachusetts Medical Society in 1956, Sosman [12] stated that his "major concern is how research affects teaching. Its present status carries dangers and threats as well as promise." Miller asked: "Do students now lose some of the wider perspective and fuller comprehension of the processes of human disease; can stimulating teaching receive its due recognition and just reward in such an atmosphere; can progress and advances in pedagogy receive essential consideration?" "By contrast with the sacred or hallowed aura surrounding the 'pure' research man, too little attention is paid and too little value attached to skill in the art of pedagogy" (Sosman [12]).

The rewards of experimental pathology are more immediate. Experimental pathology can be more appealing, more dramatic; can obtain grants readily to purchase

Submitted for publication Oct. 18, 1956.

Department of Pathology, Albert Einstein College of Medicine, Yeshiva University.

expensive equipment, and to obtain technical, clerical, and professional assistants and fellows, who are paid better than "ordinary" pathology residents. With a careful protocol and controls, an experimental pathologist has his material and daily effort immediately before him, with presentations at several scientific meetings in the offing. With success comes satisfying recognition and more rapid academic promotion.

Not so for the plodding autopsy prosector in the "lower echelon." The autopsy cannot be productive of rapid publication and rewarding achievement. At times the autopsy is smelly, unclean; it often presents trying emotional situations and demands responsibility for serious decisions, often presenting annoying legal problems. It takes diligent personal work, even physical labor, and a willingness to expose oneself to hazards of infection. It requires patient long years of training and continued application and scholarship to achieve the necessary ability to interpret an autopsy adequately. The autopsy is geared to the "hard budget," with no grants, no funds for equipment, subsidized professional and technical personnel, and travel expenses. No charges are made for the autopsy, and no income, patent profits, or "watered budget" for the college or hospital is derived from the autopsy service. This lack of financial support is a major problem confronting the autopsy service.

The autopsy service suffers from the division of departments of pathology, noted by Starr and apparently supported by Cowan,[8] into research and routine. The autopsy then invariably finds itself in the "basement" floor by location and evaluation, with an inadequate and overworked staff. The vicious cycle is completed when promotion, funds, and space allotment follow this pattern, as indeed they soon must. All members of a pathology department should have equal opportunity to do investigative work, with adequate personnel, funds, and encouragement. The stimulation of research will then benefit all alike. The stimulus of creative achievement can then reach the entire department and its teaching of pathology.

Is one justified in equating full-time "pure" research with better teaching? (Cowan[8]) "Many men who are excellent investigators make poor teachers" (Sosman[12]). The pressure to publish, i. e., "to produce," is upon the "pure" investigator, too; that is his "service load." Is the routine pursuit of simple truths valueless in a medical school; is there no place any longer in our universities for the simple, yes unproductive, scholarship, the pursuit of knowledge for its own sake, with its proper integration and stimulating transmission to the student? The autopsy is a most effective teaching instrument and can be geared to integrate and correlate the knowledge of basic sciences with clinical experience.[14] It is difficult to present autopsy findings in a stimulating manner without a positive contribution to teaching of undergraduates and graduate students. The autopsy teaches, both teacher and student alike; "we learn by teaching" (Sosman[12]).

By its very nature, the contribution of the autopsy is in another realm of probing endeavor, and cannot be replaced easily, if at all, by the controlled experiment. This constitutes a source of strength, not of weakness. It is this that makes autopsy interpretation an art, at times a difficult art, requiring a broad sphere of knowledge and extensive actual personal experience and a slow process of maturation. Can one conceive of a medical college without the full utilization of the autopsy?[14] The modern autopsy is interpreted from the functional standpoint; the analyses are now dynamic and basic, with fuller use of histo- and cytochemistry and fuller application of modern advanced instrumentation. Szent-Györgyi has put it best: "There is no real difference between structure and function; they are two sides of the same coin." The autopsy clarifies, illuminates, and gives meaning to clinical medicine.

Can the downgrading of autopsy pathology by some few experimental pathologists represent a compensation for the insecurity that follows from a doubt as to their own daily contribution to medicine and the training of medical practitioners? These few gain importance, for they control departments, sit on faculty councils, and as teachers are in a position to perpetuate their biased viewpoints. The student can hardly be expected to be stimulated to enter upon the study of autopsy pathology as a career in an atmosphere where experimental pathologists as department chairmen constantly downgrade the autopsy. Thus the vicious cycle of increasing shortage of qualified morphological pathologists and inadequate and uninspired service is further compounded and perpetuated.

The solution is "not to devaluate the autopsy, but to improve the service . . ." [and here] "money has definite therapeutic potentialities" (Cannon).[7] The recommendation that "the autopsy be simplified" to "secure information of immediate utility to the clinician"[1] is gratuitous and degrading to a field of endeavor that initiated the modern era in medicine. The pathologists must not be relegated to the role of handmaiden to the clinician, to apply his eponyms, lest epithets follow. Would it not be more appropriate for the professor of pathology who "has washed his hands of the whole business [the autopsy], and has declined to be responsible for the autopsy service,"[1] to better use his unique talents to train research workers in a research institute or in a postgraduate program, rather than to teach elementary pathology to undergraduates in a medical school? It may well be that our primary objectives in undergraduate education are due for a reexamination and reevaluation in this regard. Some confusion now exists. Keefer[17] has called for a proper balance to achieve our primary objective, "the expansion and improvement of medical education integrally related to research" in its relation to "the development of strong programs for graduate training." Relating research integrally in undergraduate medical education is essential if teaching is not to be affected adversely by having research an insular effort, no matter how outstanding it may be.

The stimulation of research by the research grant has been outstanding. Similarly, the autopsy service needs equivalent financial support and academic recognition. The teaching grant could do just that for the autopsy, extending its usefulness beyond a mere confirmation of diagnoses and clinical correlation to have it become an instrument of probing investigation and stimulating, integrating teaching. The pathologist now has little or no control of the funds that can be channeled toward the development of an excellent and outstanding autopsy service. There is never any income derived from the autopsy, and this may well be as it should. The autopsy service has always been subsidized, but only by the individual hospital and pathologist. This accounts for its precarious financial status in the face of a balanced budget, with all credit and no debit side to the ledger. This variable and inadequate support limits the scientific expansion of this important phase of pathology. Grant support would seem to be as justified as it is necessary, both as a research and as an educational effort. We trust that the present grant procedure of the U. S. Public Health Service and other public and private agencies can be expanded in this direction. The Mental Health Training Grant is a precedent that can well apply here, with equal benefit to the community.

There need be no conflict between analytical autopsy study and experimental pathology; one can complement and supplement the other (McManus).[15,16] The autopsy is not in competition with experimental laboratory investigation; it cannot supersede it; yet it can make a distinct contribution by helping to initiate, promote, and stimulate the application of the experimental method to its morphological findings and lack of findings alike (functional component).

Grant support of autopsy investigation will remove the existing financial hurdle, which is now the handicap to the development of this aspect of pathology. The problem is more than that of survival of the autopsy procedure, and surely not one of its further pragmatic simplification; rather, it is that of expanding its usefulness. To achieve this, the routine autopsy must be made more probing by the application of every means of study and analysis at our disposal. It is thereby that the research potential of pathology can be enhanced, not in a new direction by artificial superimposed fiat, but by stimulating its unique inherent worth and true purpose in a fundamental manner. The contribution of an autopsy can be just as stimulating as the application of the $\chi^2$ test to "blindfold" test data, as analytical as the statistical multicolored graph, and as informative too as the diagrammatic presentation of the electron shifts in hemoglobin.

## Conclusions

There should be no controversy about the autopsy and its contribution to medical education. The autopsy remains invaluable in medical teaching generally, and indispensable for instruction in pathology.

With no other means of financing the expansion of its study now available, the autopsy requires and deserves grant support, similar to the successful aid programs for experimental research and other teaching efforts. Financial support of the autopsy will elevate this procedure scientifically and help the teaching and all research programs in our medical colleges, promoting the "integral relationship" and cross-fertilization of research and teaching.

Let us not discount this proved procedure, but, rather, exploit the opportunity it offers for the unified teaching we are now seeking. Let us direct our efforts to learn to use the full potential of the routine autopsy and to promote its greater use in medical education and in research.

Albert Einstein College of Medicine, Eastchester Rd. and Morris Park Ave. (61).

## REFERENCES

1. Starr, I.: Potential Values of the Autopsy Today, Guest Editorial, J. A. M. A. 160:1144-1145, 1956.

2. Burn, C.: The Autopsy, Correspondence, J. A. M. A. 161:175, 1956.

3. Bohrod, M. C.: The Autopsy, Correspondence, J. A. M. A. 161:176-177, 1956.

4. Barnett, R. N.: The Autopsy, Correspondence, J. A. M. A. 161:177, 1956.

5. Lev, M.: The Autopsy, Correspondence, J. A. M. A. 161:177, 1956.

6. Enterline, H. T.: The Autopsy, Correspondence, J. A. M. A. 161:749, 1956.

7. Cannon, P. R.: Clinical Lessons Learned at the Morgue, Guest Editorial, J. A. M. A. 161:730-732, 1956.

8. Cowan, D. R.: The Autopsy, Correspondence, J. A. M. A. 161:750-751, 1956.

9. Klemperer, P.: The Autopsy, Correspondence, J. A. M. A. 161:175-176, 1956.

10. Karsner, H.: The Autopsy, Correspondence, J. A. M. A. 161:176, 1956.

11. Medical Research: A Midcentury Survey, American Foundation Studies, Boston, Little, Brown & Company, 1955.

12. Sosman, M. C.: Annual Discourse: *Docendo Discimus*, New England J. Med. 254:1105-1112, 1956.

13. Miller, G. E.: Adventure in Pedagogy, Section Medical Education, American Federation for Clinical Research, Atlantic City, April 29, 1956.

14. Angrist, A.: Effective Use of the Autopsy in Medical Education, J. A. M. A. 161:303-309, 1956.

15. McManus, J. F. A.: Progress in Fundamental Medicine, Philadelphia, Lea & Febiger, 1952.

16. McManus, J. F. A.: The Autopsy, Correspondence, J. A. M. A. 161:749-750, 1956.

17. Keefer, C. S.: Medical Education Tomorrow, New England J. Med. 255:650-655, 1956.

# Pulmonary Arteriosclerosis Due to Oxygen, Nitrogen, and Argon Embolism

*Experimental Study*

P. J. BARNARD, M.D., Pretoria, Union of South Africa

When blood clot is chopped fine and repeatedly injected intravenously into rabbits, arteriosclerosis of the lungs will arise.[1-5] This will happen whether a rabbit receives clots prepared from its own blood or blood from another animal and also whether red blood clot or fibrin clot is used. If changes due to canalization of clot are left out of account, the main lesions caused by recurrent thromboembolism are endarteritis obliterans and fibroelastosis of the muscular pulmonary arteries. Pulmonary arteries of elastic type outside the lungs usually escape injury, but they sometimes display small fibrous plaques in the intimal coat. Atherosclerosis does not occur. Provided that fragments of blood clot are injected often enough, pulmonary hypertension and cor pulmonale will arise in addition to the arteriosclerosis. Thromboembolic arteriosclerosis has also been recognised in man,[6] but it was postulated before this.[7,8] It thus seems established that some cases hitherto taken for "primary" pulmonary arteriosclerosis or Ayerza's disease are due to latent embolism of the lungs by small blood clots.

It has up to now been assumed that thromboembolic arteriosclerosis is due to organization of blood clot into the intimal coat of pulmonary arteries. To test this, 10 rabbits were given single large doses of air intravenously for one to two months. Air was chosen to avoid organization, a process which adds complexity if solid emboli are used. It was found that arteriosclerosis like that of thromboembolism arose.[9] It will be here demonstrated that embolic arteriosclerosis is largely independent of the kind of emboli used, for it may be produced as readily by repeated injections of oxygen, nitrogen, and argon as by injections of thrombi and air.

Submitted for publication Aug. 8, 1956.

Department of Morbid Anatomy, Institute for Pathology, University of Pretoria.

## Material and Methods

Sixty young adult rabbits were divided into three groups, each comprising 10 males and 10 females. Animals of these three groups were repeatedly injected with oxygen, nitrogen, and argon into their ear veins, using 26-gauge needles to obtain small bubbles in the blood stream. As a start each rabbit received 0.1 cc. of the appropriate gas per kilogram of body weight each day for three days. This initial dose was then raised by 0.05 cc. per kilogram and given each day for three days. Each succeeding three days after this the dose was raised again by 0.05 cc., injections being given daily as before. After 30 single daily doses had been administered to each rabbit, three males and two females were killed at random from each group by giving magnesium sulfate solution intravenously. After another 30 doses two more males and three females from each group were killed. Because four rabbits had died between the 65th and the 81st injections, and to avoid losing any more at this stage, the dosage was stepped down by 0.05 cc. per kilogram after each succeeding three days. When each of the remaining animals had been given 120 doses, they were killed to end the experiment. The commercially prepared gases used were about 96% pure; this concentration was thought to be satisfactory, as even the largest dose employed, 1.4 cc. per kilogram, could have contained traces only of atmospheric gases other than the one selected for injection.

The following tissues of each rabbit were examined microscopically: lungs, heart, thoracic

aorta, main pulmonary artery, bronchi, trachea, thyroid gland, esophagus, liver, spleen, kidneys, adrenals, pancreas, retroperitoneal fat, tongue, and testes or ovaries. All lung sections were stained with hematoxylin and eosin and the Weigert-Van Gieson method, but all others with the latter only. As needed, lung sections were also stained with the periodic acid-Schiff reagent or for iron by Perls' method.

In the last 15 animals of each group the abdominal aorta was cannulated and, without opening the chest, a lead phosphate-agar mass [9] run in. This was done to identify bronchial arteries in sections, because the recurrent gas embolism had caused hypertrophy of intrapulmonary arteries in the first five animals of each group so as to make them look like systemic arteries. Since this heavy mass also filled the coronary arteries, the hearts were not weighed, but right ventricular hypertrophy had taken place in many animals from each group as judged with the naked eye.

Twenty-five normal rabbits of mixed breeds kept with those experimented upon served as controls. As an additional control, the ear veins of five of these were merely punctured on 30 daily occasions, using the same technique, syringes, and needles as those employed to inject the gases.

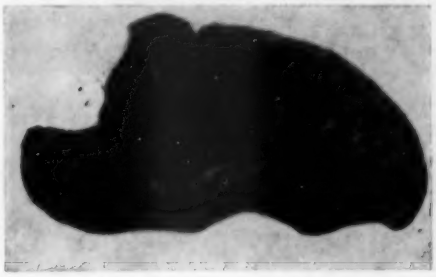
## Results

*Lungs.*—These had normal pleural surfaces, but thickened blood vessels stood out on their cut surfaces (Fig. 1).

(*a*) Lesions of lung arteries were of similar types irrespective of the gas used, but on the whole were less severe in the rabbits which received injections of oxygen than in those which got nitrogen or argon.

(*b*) Arterial lesions were as pronounced after 30 injections in a few rabbits of each group as in some others of the same or other groups after more than 30 injections.

Fig. 1.—Left lung showing thickened intrapulmonary arteries dotting the cut surface. Argon 11; female; 82 injections. About natural size.



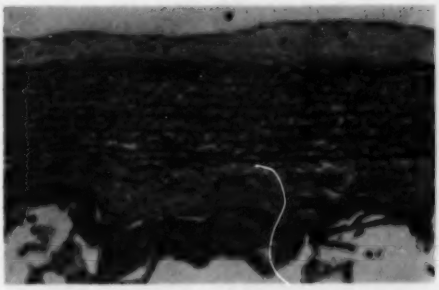


Fig. 2.—Intrapulmonary elastic artery with mucoid intimal thickening. Nitrogen 11; female, 30 injections. Weigert-Van Gieson stain; × 144.

(*c*) Progress of lesions after 60 injections was very slow, for their severity was only slightly less than after 120 injections.

The pulmonary artery and its branches outside the lungs were normal. Of its intrapulmonary branches, *elastic arteries* (those of external diameter greater than 1.0 mm.) were involved in about a quarter of the animals. They showed fibrous or fibroelastic intimal thickenings which usually encircled the lumen. A few lesions consisted of scanty fibroblasts set in ground substance faintly positive to periodic acid-Schiff staining (Fig. 2). *Large muscular arteries* (of external diameter 0.1 to 1.0 mm.) were the most frequently and severely affected. Pathological change was almost restricted to these vessels in a few animals of each group, small muscular branches of abnormal parent stems remaining intact (Figs. 3 and 4). Arteritis, intimal fibrosis, damage to elastic tissue, and medial hypertrophy were the chief lesions encountered. *Arteritis* was characterized by hyperplasia of endothelial cells and heavy infiltrates of neutrophils, but with lymphocytes, large mononuclear cells, and plasma cells present as well. Neutrophils were far less numerous in the medial than in other coats (Fig. 5). How much this infiltration by neutrophils promoted the arteriosclerosis found is impossible to say, but it left adventitia intact, to judge from lack of fibrosis around vessels, the intimal coats of which were fibrosed. Arteritis was

Fig. 3.—Large muscular artery. Intima is folded; endothelial nuclei are radial, and tunica media is grossly hypertrophied with foci of myocytolysis. The small muscular branch is intact. Nitrogen 8; male; 30 injections. Weigert-Van Gieson stain; × 144.

Fig. 4.—Large muscular artery showing folded intimal outline, fibrosed intima with radially directed nuclei, hypertrophied media, and inflammatory-cell infiltration of the adventitia. The small muscular branch is intact. Oxygen 3; male; 30 injections. Weigert-Van Gieson stain; × 144.

restricted to the lungs, as in embolism by fragmented blood clot and air. It was not due to contamination, as injections were given aseptically and suppuration did not occur. *Intimal fibrosis* was usually pronounced, both in degree and in extent. Fibrosis encircled the lumen as a rule (Figs. 4, 5, and 6) and rarely affected only a segment of it. Lesions of the former kind are here called circumferential intimal fibrosis, and of the latter kind, eccentric intimal fibrosis. Collagen in fibrosed intima was disposed both around the lumen and radial to it. Where radial, the commoner arrangement, endothelial nuclei were similarly directed, and the intimal contour was con-

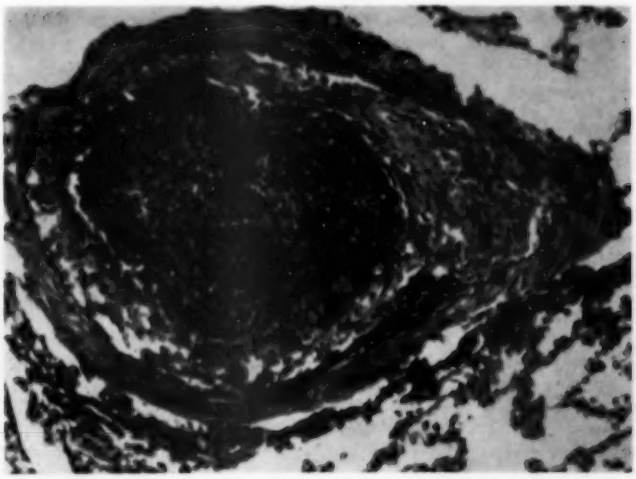


Fig. 5.—Large muscular artery showing inflammatory-cell infiltration of intima and adventitia. Proliferated intimal cells surround and infiltrate the blood clot. Argon 1; male; 30 injections. Weigert-Van Gieson stain; × 144.

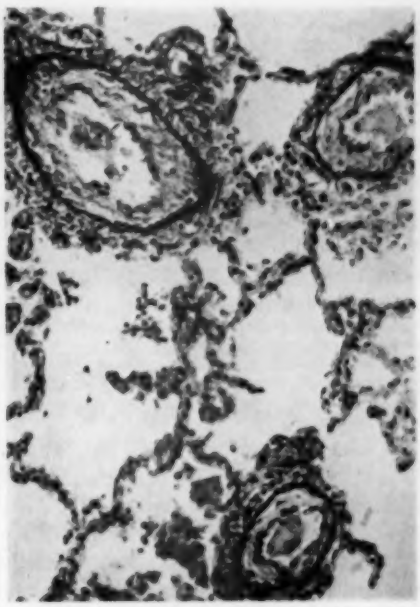


Fig. 6.—Smooth circumferential intimal fibrosis. Field of three arteries with intimal fibrosis of smooth outline and leukocytic infiltration of their adventitial coats. Medial coats are not hypertrophied. Argon 3; male; 30 injections. Weigert-Van Gieson Stain; × 144.

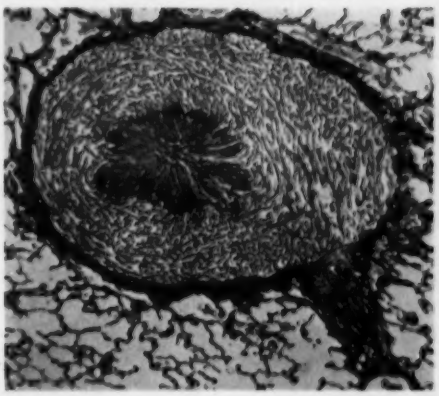


Fig. 7.—"Vasospastic arteriosclerosis." Large muscular artery with lumen almost obliterated, contorted intimal outline, intact internal elastic lamina, and hypertrophied media. Oxygen 10; male; 60 injections. Weigert-Van Gieson stain; × 53.

torted (Figs. 4, 7, and 8). At points of branching, the lumen was in some arteries changed into cavernous blood spaces. These did not seem to have arisen from canalized blood clot, as their walls contained no red blood cells or hemosiderin, and thrombi are hardly found at all in gas embolism. This suggests that where recanalizations are

Fig. 8.—"Vasospastic arteriosclerosis." Lumen contorted and almost obliterated, intimal fibrosis with radially directed collagen and cellular nuclei, intact internal elastic lamina, thickened media, and leukocytic infiltration of the adventitial coat. Nitrogen 18; female; 120 injections. Weigert-Van Gieson stain; × 144.

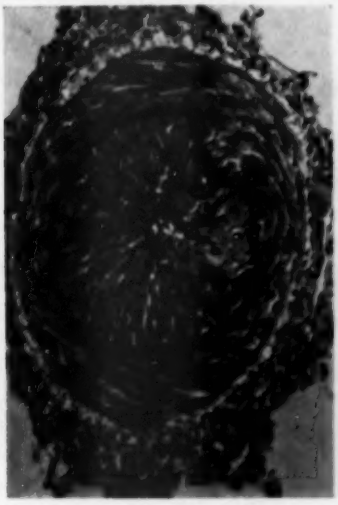


Fig. 9.—Point of transition between arteries of elastic and muscular types showing canalized fibrous intima of polypoid aspect and ruptures of the internal elastic lamina. Argon 4; male; 30 injections. Weigert-Van Gieson Stain; × 144.

found, even in thromboembolic arteriosclerosis, they need not be attributed to organization of blood clot. In some arteries stretches of fibrosed intima were excavated by endothelium so as to cause polypoid formations (Fig. 9), a change encountered in elastic arteries as well. Such formations were very occasionally seen to lie on top of one another, younger tissue surmounting older, as if separate vascular insults had taken place. This was also seen in fibrosed intima not channeled by endothelium. Transmedial vascularization [11] arose only in the largest muscular arteries, where thickening of intima was greatest. *Damage to elastic tissue* took various forms, thickening and rupture of the internal lamina being often found (Figs. 9 and 10). New elastic tissue seldom had formed within fibrosed intima, and if it did, in small amounts only. Duplication of the internal lamina was unusual. In a rabbit which had received 81 injections of argon, no arteriosclerosis could be found, but the medial coat of its large muscular arteries had hypertrophied greatly. *Medial hypertrophy* (Figs. 3 and 7) involved large muscular arteries in all animals, but the small muscular arteries of some in each group escaped this change. Muscle fibers in the medial coat of some vessels showed paranuclear vacuolation, and in others they seemed to have undergone lysis (Fig. 3), like that described in human heart muscle.[12] Scarring was met with in some arteries but only where the internal elastic lamina had ruptured (Fig. 10). Lesions of *small muscular arteries* (of external diameter less than 0.1 mm.) were of the same kinds as those of large muscular arteries but were fewer in number as a rule and less advanced. A greater proportion of circumferential and eccentric intimal fibroses was seen, but folded encircling lesions with a tendency to radial disposition of collagen and endothelial nuclei, like that of Figures 7 and 8, were also common. Atherosclerosis was not found, and the bronchial arteries were normal. *Precapillaries*, which are large-caliber endothelial tubes surrounded by an elastic membrane of varying completeness, were in a few

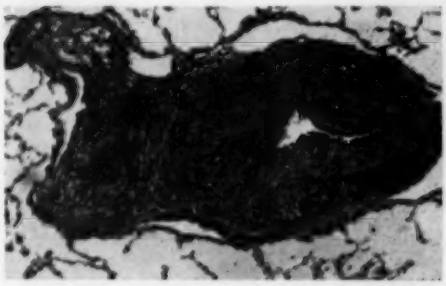


Fig. 10.—"Vasospastic arteriosclerosis." Internal elastic lamina ruptured at 12 o'clock, with fibrosis extending into the medial coat. At top left the lumen of a small branch is canalized. Nitrogen 17; female; 120 doses. Weigert-Van Gieson stain; × 67.

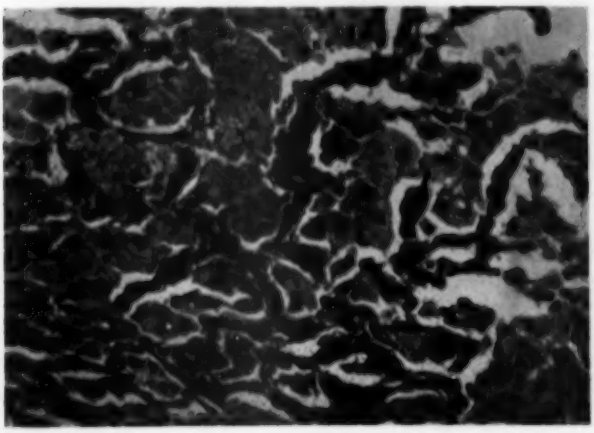


Fig. 11.—Interstitial pneumonia. Alveolar walls are thickened by endothelial hyperplasia, and alveolar spaces are filled with lipophages. Nitrogen 9; male; 30 injections. Weigert-Van Gieson stain; × 220.

animals involved by conversion of their endothelium into a substance reacting pale pink to Van Gieson's stain. *Capillary* endothelial cells had multiplied in scattered places so as to cause thickening of alveolar walls. This was usually accompanied by infiltrates of neutrophils and lymphocytes, and in some areas by cuboidal metaplasia of alveolar walls and accumulation within alveolar spaces of lipophages in large numbers (Fig. 11). Interstitial pneumonia like this, described in greater detail elsewhere,[4] also occurs after embolism by air and thrombi and is evidently harmless, as fibrosis of alveolar walls is not seen. This lesion, it is suggested, resulted from flow into capillaries of inflammatory products from arteries damaged by embolism, which vessels had themselves responded to injury by arteritis.

*Other Tissues.*—Of the very large number of vessels examined in other tissues, only some small intramyocardial arteries showed intimal fibrosis, but no more so than in controls. This was also true for those few hearts with interstitial infiltrates of lymphocytes and large mononuclear cells and small foci of myocardial atrophy. Spontaneous myocarditis is common in rabbits.[13] Mild fatty change of myocardium, as of the liver, was found in a few animals and was ascribed to the episodes of anoxemia that embolism had produced.[14,15] Slight subacute and healed pyelonephritis was not infrequent, but not more so than in controls. The arteries of all organs other than of lungs and heart were normal.

## Comment

Differences between the lesions of recurrent thromboembolism and those of gas embolism were found to be of degree and not of kind. Medial hypertrophy obtrudes less after injections of fragmented clot, probably because its organization is associated with greater damage to the media, and because in no published report have thrombi been injected so often or in such large amounts as were gases in the experiment here recorded. Also, thromboembolism elicits far more new elastic tissue in intimal thickenings. This difference suggests that blood clot might contain a principle which stimulates the production of elastic tissue.

Arteriosclerosis of the lungs from thromboembolism is different when a rabbit receives only one large dose of clot fragments or many doses. If the animal is killed some weeks after a single dose, segmental fibrosis or fibroelastosis is found. By contrast, such lesions surround the lumen after repeated doses. To the segmental lesion of thromboembolism the name "thrombogenic arteriosclerosis" might be given. This kind of arteriosclerosis, since it lacks lipoid deposits, must not be confused with the thrombogenic atherosclerosis held to involve systemic arteries.[16] The circumferential lesions of thromboembolism, on the other hand, seem to arise otherwise than by incorporation of blood clot, because air embolism also produces them,[9] as also do emboli of oxygen, nitrogen, and argon, as is here shown.

Since embolism produces anoxemia, it might be thought that lack of oxygen caused the arteriosclerosis. But arteriosclerosis is not regularly found in congenital heart disease, even in cases of prolonged cyanosis. In such cases it could be argued that pulmonary arteries are "accustomed" to deficiency of oxygen and that it might be otherwise if chronic cyanosis is acquired. There is as a rule, however, no noteworthy arteriosclerosis in the muscular arteries of emphysematous lungs, even with cyanosis severe enough to have caused cardiac hypertrophy and failure. The same is true for rats and mice made to breathe for long periods air the oxygen tension of which had been greatly reduced.[20] Also, since oxygen shares with thrombi, air, nitrogen, and argon the ability to induce arteriosclerosis, it seems improbable that oxygen to excess in the form of gas bubbles had exerted specific harmful action.

It may be that different kinds of emboli possess in common a means of disturbing arterial nutrition not specifically due to lack

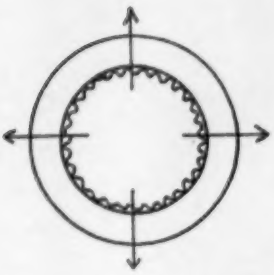


Fig. 12.—Normal muscular artery showing thin medial coat. Arrows indicate direction of pressure gradient across the wall.

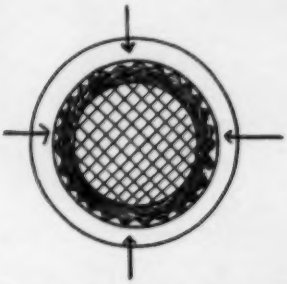
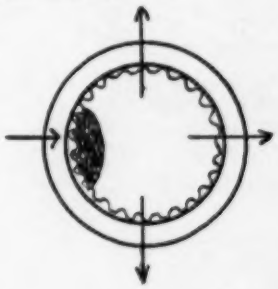


Fig. 13.—Smooth circumferential intimal fibrosis due to a substance excluding blood from the lumen. Arrows indicate that nutrition of media is maintained by reversal of the pressure gradient: "obstructive intimal fibrosis."

of oxygen. Muscular arteries as small as those of rabbit lung, because they have no vasa vasorum, require for their nutrition and removal of metabolic waste the outward seepage of lymph through tissue spaces of their walls. This seepage occurs outward from the arterial lumen because blood pressure here is greater than in capillaries of the adventitia.[17] Flow of nutritive lymph can conceivably become embarrassed locally in five different mechanical ways alone or in combination: substances occupying vessel lumens to the exclusion of blood, collapse of vessels beyond sites of occlusion, vasospasm, overstretching of vessel walls, and fibrosis of the adventitia.

*Substances occupying vessel lumens to the exclusion of blood* will cut off lymph to the intimal coat. Occluding blood clot and gas bubbles thus impair intimal nutrition right around the lumen along segments of varying length. The effects of such impairment might conceivably extend beyond the limits of any occluding substance because blood ceases to flow proximally and because the lumen empties distally. These considerations apply only if collateral circulation becomes insufficient, as is likely to happen where showers of blood clot or gas bubbles impact in pulmonary vessels. Tissue response to impaired nutrition varies according to whether it is partial or complete. If complete, all cells die. If partial, as happened here because infarction did not occur, connective tissue cells are stimulated to multiply. With complete obstruction, the pressure gradient moving lymph across vessels containing no flowing blood would reverse and so explain why intima becomes fibrosed and why the media tends to stay intact. If, therefore, a vessel is occluded, intimal cells will proliferate and eventually lay down collagen of uniform thickness around the lumen because nutrition is about equally impaired at all points. This is one way in which *circumferential intimal fibrosis* of smooth outline might come about (Figs. 5 and 6; cf. Figs. 12 and 13). If, on the other hand, a substance should lie against intima, not shutting off blood flow entirely, only a segment of the luminal circumference would be affected. Small blood clots fulfill this condition ideally, for if lysis were to fail, they often shrink to one side and undergo organization to cause *thrombogenic or eccentric intimal fibrosis* (Fig. 14).

Fig. 14.—Eccentric intimal fibrosis, such as might occur from organization of blood clot: thrombogenic arteriosclerosis.



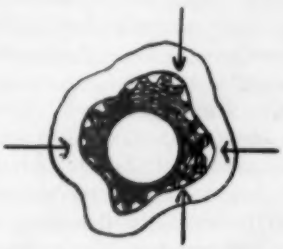


Fig. 15.—"Collapse intimal fibrosis": lumen empty, circumferential intimal fibrosis, media wavy, and pressure gradient reversed.

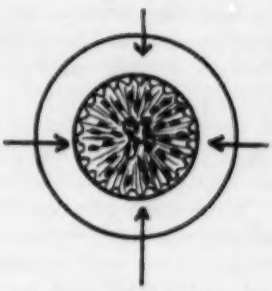


Fig. 16.—"Vasospastic intimal fibrosis": intimal outline contorted, lumen small, collagen and intimal nuclei radial, and media thick.

*Collapse of vessels beyond sites of occlusion* might be expected to cause lesions of another histological type. If normal arteries of man and animals are empty of blood when doubly ligated and divided, it is seen on subsequent examination that the whole wall has become wavy (Mehrotra,[18] Fig. 2; cf. Fig. 15). Such vessels in the living animal must rely for nutrition upon movement of lymph from the adventitia, again leaving intima at greater distance than media from a source of nutrition. Because of this, intima will fibrose before the media atrophies. This is one way it seems in which the intimal fibrosis of "disuse" might arise. Lesions of the type ascribable to collapse and disuse were, however, not found in the experiments here described.

*Vasospasm* might also disturb the nutrition of arteries so as to cause intimal fibrosis without at first harming the media. There are two local conditions affecting small muscular arteries in which spasm is known to precede circumferential intimal fibrosis (endarteritis obliterans). They are chronic ergot poisoning and the Raynaud phenomenon. Arterial lesions from cases of these conditions have distinctive features, to judge from illustrations (Yater and Cahill,[19] Figs. 3 and 6; Richards,[20] Fig. 55). These features are circumferential intimal fibrosis of contorted outline with tendency to radial orientation of collagen fibers and intimal nuclei. Medial hypertrophy is sometimes a third feature, at least in pulmonary arteries (Figs. 4, 7, and 8; cf. Fig. 16). Comparison of arteries in ergot poisoning, the Raynaud phenomenon, and the kind of embolic lesion here illustrated as "vasospastic" suggests that spasm causes intimal fibrosis of a type distinctive enough to prompt the term "vasospastic arteriosclerosis." The comparison also suggests that orientation of intimal cells determines the direction in which collagen fibers are laid down. According to Buck[21] orientation of fibroblasts in regenerating tendon likewise seems to direct the collagen fibers produced. Spasm of arteries, even if blood continued to dribble through them, would embarrass mural nutrition by compressing tissue spaces and by increasing diffusion distance from bunching together cells both in intima and in media. The effect of arterenol (noradrenaline; Levophed) on arteries of the skin reinforces the concept of vasospastic arteriosclerosis.[9] If arterenol should leak from a vein during treatment for hypertension, ulceration might arise, for arteries of the part are thrown into prolonged spasm. In a case of this kind[9] intimal fibrosis of contorted outline with radial vasospasm participates in causing embolic collagen was found. The assumption that arteriosclerosis therefore seems warranted, the more so because it was here found that intimal fibrosis of "vasopastic" type reached greatest development in just those pulmonary arteries with the thickest muscular walls. Since the walls of normal pulmonary arteries are about one-fifth as thick as those of their systemic counterparts,[22]

it could be held that they are too weak to contract hard enough for arteriosclerosis to arise. However, in rabbit anaphylaxis pulmonary arteries constrict so strongly that blood flow through them is cut off and the animal dies of acute cerebral ischemia.[28] But it has been found that adenosinetriphosphate, a substance that constricts pulmonary arteries selectively,[24] fails to cause arteriosclerosis even with repeated high sublethal doses.[9] This may be because adenosinetriphosphate produces spasm too fleeting for mural nutrition to suffer. If high mural tension is the cause of dyspnea from embolism, as seems likely,[25] it is easier to grasp why adenosinetriphosphate fails to elicit pulmonary arteriosclerosis. Following a large dose of this substance dyspnea is slight and short-lived, whereas after large doses of emboli dyspnea is severe and often prolonged for up to half an hour. From the observation that man and animals react to anoxia with pulmonary hypertension,[26,27] it has been inferred that lack of oxygen causes pulmonary vasoconstriction. If continuous vasospasm indeed helped to cause arteriosclerosis in the experiment made here, it would be anticipated that animals subjected to prolonged anoxia would also develop this condition, but they do not. Failure of adenosinetriphosphate and chronic anoxia to cause arteriosclerosis experimentally need not, however, disprove the concept of vasospastic arteriosclerosis, until it is shown that they induce severe sustained vasoconstriction.

*Overstretching of vessel walls* by embolic hypertension might possibly aid development of arteriosclerosis by pressing elements of the arterial wall hard against the more unyielding adventitia, with compression of tissue spaces as a result. This could conceivably retard lymph flow sufficiently for fibrosis to occur in spite of raised pressure in the lumen. Supposing the wall to be equally strong at all points, the diagnostic criteria of arteriosclerosis from this cause would be smooth circumferential intimal fibrosis, straightening or rupture of elastic laminae, and dilatation with atrophy of the media (Fig. 17). If, on the other hand, the wall were to be weaker at one point than another, these changes could be segmental. In either case large molecular substances like $\beta$-lipoprotein would tend to accumulate and produce the features of atherosclerosis in addition. No lesions of this kind were seen here, but overstretching might be one way in which hypertension promotes development of arteriosclerosis, particularly in elastic arteries, as their walls

Fig. 17.—"Distention or pulsion intimal fibrosis": smooth intimal fibrosis, internal elastic lamina ruptured or straight, media thinned or fibrosed. Broken arrows indicate retarded flow of lymph. Once intima has fibrosed, lymph flow will tend to reverse.

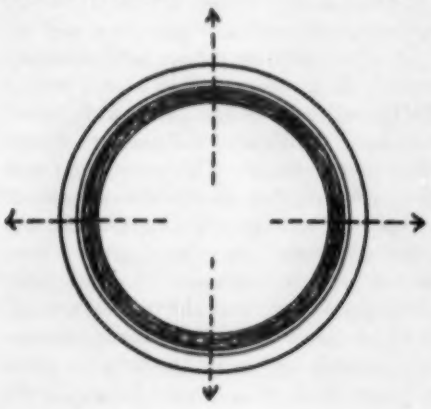
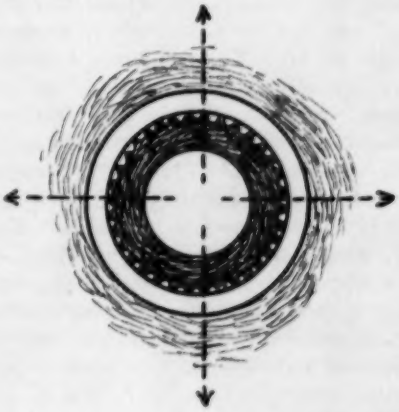


Fig. 18.—"Malabsorption intimal fibrosis," from sclerosis of the adventitia: Broken arrows indicate retarded lymph flow. Once intima has fibrosed, flow will tend to reverse.



are less able to withstand distending forces than those of muscular arteries. That atherosclerosis failed to occur was expected because the rabbit is not susceptible to this condition.

*Adventitial fibrosis*, by grossly retarding lymph flow through arterial walls, might also be anticipated, if the mechanical hypothesis of arteriosclerosis here propounded applies, to cause intimal fibrosis (Fig. 18). Such fibrosis would be segmental or circumferential, depending upon how far fibrosis happens to have extended around the vessel wall. Adventitial fibrosis is not a feature of embolic arteriosclerosis and does not, therefore, help to account for the pathogenesis of lesions here described.

The mechanical view of how arteriosclerosis arises, as discussed and illustrated, may be extended to cover other lesions also, medionecrosis, for instance. It has here been developed, from the restricted application of mechanical considerations by Rotter[28] and de Langen,[17] into a unitarian hypothesis for all the otherwise confusing variety of lesions seen in arteriosclerosis. Substances preventing contact of blood with intima and vasospasm are, of the five ways in which arterial nutrition might be impaired mechanically, the most likely to participate in embolic arteriosclerosis. Other experiments aimed at testing the unitarian hypothesis here stated are now in progress.

## Conclusions

Repeated intravenous injections of oxygen, nitrogen, and argon into separate groups of rabbits cause arteriosclerosis of the lungs. Arterial changes so produced are of similar kind irrespective of the gas used. Since arterial lesions from embolism by these gases of the atmosphere resemble those of embolism by blood clot fragments, thromboembolic arteriosclerosis is mainly due to some factor other than incorporation of blood clot into arterial walls. Impaired nutrition of arteries caused by substances occupying their lumen to the exclusion of blood, and vasospasm are suggested to be the chief means by which embolic arteriosclerosis is brought about.

The South African Council for Scientific and Industrial Research defrayed the costs of this work; Prof. J. Barnetson helped; my wife, a stipendiary of the Council, made the slides and diagrams and took the photographs; the University of Pretoria paid for reprints, and Mr. P. J. Jacobs assisted with the animals.

Institute for Pathology, University of Pretoria.

## REFERENCES

1. Harrison, C. V.: Experimental Pulmonary Arteriosclerosis, J. Path. & Bact. 60:289-293 (April) 1948.

2. Muirhead, E. E., and Montgomery, P. O'B.: Thromboembolic Pulmonary Arteritis and Vascular Sclerosis: Its Experimental Production in Rabbits by Means of Intravenously Injected Human Amniotic Fluid and Autogenous Blood Clots, A. M. A. Arch. Path. 52:505-517 (Dec.) 1951.

3. Heard, B. E.: Experimental Study of Thickening of Pulmonary Arteries of Rabbits Produced by Organisation of Fibrin, J. Path. & Bact. 64:13-19 (Jan.) 1952.

4. Barnard, P. J.: Experimental Fibrin Thrombo-Embolism of the Lungs, J. Path. & Bact. 65:129-136 (Jan.) 1953.

5. Barnard, P. J.: Pulmonary Arteriosclerosis and Cor Pulmonale Due to Recurrent Thromboembolism, Circulation 10:343-361 (Sept.) 1954.

6. Owen, W. R.; Thomas, W. A.; Castleman, B., and Bland, E. F.: Unrecognized Emboli to Lungs with Subsequent Cor Pulmonale, New England J. Med. 249:919-926 (Dec. 3) 1953.

7. Goedel, A.: Zur Kenntnis der Hypertrophie des rechten Herzens und schwerer Kreislaufstörung infolge Verödung der Lungensschlagaderperipherie, Arch. path. Anat. 277:507-521, 1930.

8. Belt, T. H.: Late Sequelae of Pulmonary Embolism, Lancet 2:730-733 (Sept. 30) 1939.

9. Barnard, P. J.: Pathogenesis of Experimental Thrombo-Embolic Pulmonary Arteriosclerosis, J. Path. & Bact., to be published.

10. Schlesinger, M. J.: Injection Plus Dissection Study of Coronary Artery Occlusions and Anastomoses, Am. Heart J. 15:528-568 (May) 1938.

11. Geiringer, E.: Intimal Vascularisation and Atherosclerosis, J. Path. & Bact. 63:201-211 (April) 1951.

12. Schlesinger, M. J., and Reiner, L.: Focal Myocytolysis of the Heart, Am. J. Path. 31:443-459 (May-June) 1955.

13. Miller, C. P.: Spontaneous Interstitial Myocarditis in Rabbits, Quart. J. Med. 13:129-147 (Jan.) 1920.

14. Sanders, J. H., and Isoe, I. M.: Intravenous Oxygen and Pulmonary Embolism, Ann. Surg. 126:208-214 (Aug.) 1947.

15. Weston, R. E., and Karel, L.: Influence of Denitrogenation on Response of Anesthetized Dogs to Intravenously Injected Oxygen, J. Clin. Invest. 26:837-849 (Sept.) 1947.

16. Duguid, J. B.: Thrombosis as a Factor in Pathogenesis of Coronary Atherosclerosis, J. Path. & Bact. 58:207-212 (April) 1946.

17. de Langen, C. D.: The Pressure Gradient in the Arterial Wall and the Problem of Arteriosclerosis, Proc. Koninkl. Nederl. Akad. Wetenschappen, s. C 56:393-396, 1953; Cardiologia 22:315-319, 1953.

18. Mehrotra, R. M. L.: Experimental Study of Changes Which Occur in Ligated Arteries and Veins, J. Path. & Bact. 65:307-313 (April) 1953.

19. Yater, W. M., and Cahill, J. A.: Bilateral Gangrene of Feet Due to Ergotamine Tartrate Used for Pruritus of Jaundice: Report of a Case Studied Arteriographically and Pathologically, J. A. M. A. 106:1625-1631 (May 9) 1936.

20. Richards, R. L.: The Peripheral Circulation in Health and Disease: A Study in Clinical Science, Edinburgh, E. & S. Livingstone, Ltd., 1946, pp. 79-81.

21. Buck, R. C.: Regeneration of Tendon, J. Path. & Bact. 66:1-18 (July) 1953.

22. Brenner, O.: Pathology of Vessels of Pulmonary Circulation, Arch. Int. Med. 56:211-237 (Aug.) 1935.

23. Gladstone, G. P., in Lectures on General Pathology, edited by H. W. Florey, London, Lloyd-Luke (Medical Books), Ltd., 1954, p. 455.

24. Emmelin, N., and Feldberg, W.: Systematic Effects of Adenosine Triphosphate, Brit. J. Pharmacol. 3:273-284 (Dec.) 1948.

25. Dunn, J. S.: The Effects of Multiple Embolism of Pulmonary Arterioles, Quart. J. Med. 13:129-147 (Jan.) 1920.

26. von Euler, U. S., and Liljestrand, G.: Observations on Pulmonary Arterial Blood Pressure in Cat, Acta physiol. scandinav. 12:301-320, 1946.

27. Nisell, O. I.: Action of Oxygen and Carbon Dioxide on Bronchioles and Vessels of Isolated Perfused Lungs, Acta physiol. scandinav. (Supp. 73) 21:1-62, 1950.

28. Rotter, W.: Über die Bedeutung der Ernährungsstörung, insbesondere des Sauerstoffmangels für die Pathogenese der Gefässwandveränderungen mit besonderer Berücksichtigung der "Endarteritis obliterans" und der "Arteriosklerose": Zugleich ein Beitrag zum Entzündungsproblem, Beitr. path. Anat. 110:46-102, 1949.

29. Barnard, P. J.: Unpublished observations.

# Multilobulated Spleen in Association with Congenital Heart Disease

*Report of a Case*

S. DONALD GREENBERG, M.D., Houston, Texas

The occurrence of malformations of other structures in patients with anomalies of the heart and great vessels is not rare. Associated anomalies of the spleen are sometimes encountered. A recent report by Ivemark [3] is concerned with these associated splenic anomalies and includes a review of the world literature on the subject.

As a result of Ivemark's article, we became interested in the relation of such a rare necropsy finding as a multilobulated spleen to the development of congenital heart disease. A survey of the necropsy material at Jefferson Davis Hospital accumulated during the years 1950 through 1955 revealed one such case in which a multilobulated spleen was associated with cor triloculare biventriculare, along with situs inversus of the stomach, pancreas, spleen, and intestine. During this same period there were 630 necropsy studies on children 10 years of age and less. Forty-eight of these patients had congenital cardiac anomalies. Among these 48 patients there were 15 who had conotruncus anomalies of the heart.

## Report of Case

This 2-day-old Negro boy was admitted to Jefferson Davis Hospital on July 25, 1955, because of dyspnea and lethargy. He had been born in a private clinic, apparently after an uneventful labor, and no immediate neonatal distress was noted. He was the product of the eighth pregnancy of his mother. One sibling had died at the age of one year, of unknown causes.

Submitted for publication Sept. 8, 1956.

From the Departments of Pathology, Baylor University College of Medicine and Jefferson Davis Hospital.

Physical examination on admission revealed no gross anomalies by inspection. The skin was faintly icteric. The infant appeared to be in no acute distress. He weighed 2380 gm. Rectal temperature was 100.2 F; pulse rate was 120 a minute, and respirations were 36. The lungs were clear to examination. The cardiac rhythm was regular. Along the left sternal border there was a Grade 2, blowing systolic murmur, which was most marked in the left third interspace and which radiated to the left axilla. There was a loud, snapping second pulmonic sound. The remainder of the physical examination was not unusual.

Urinalysis was within normal limits. The white blood cell count was 10,800 per cubic millimeter. Hemoglobin was 11.9 gm. per 100 cc. The serologic test for syphilis (VDRL) was negative. Serum bilirubin was 3.1 mg. per 100 cc. A direct Coombs test was negative. A roentgenogram of the chest revealed some widening of the mediastinal shadows, presumed to be due to the thymus. An apparent air bubble in the right upper quadrant suggested transposition of the viscera. The admission diagnosis was "sepsis, etiology undetermined."

Therapy included intravenous fluids, penicillin, and sulfisoxazole (Gantrisin). A white blood cell count on the third hospital day was 14,600 per cubic millimeter, with 49% neutrophilic granulocytes. Staphylococcus albus was cultured from the umbilicus. Tetracycline U. S. P. (Achromycin) was added to the therapy on the fourth hospital day. Six days after admission the patient seemed improved. He was afebrile, and his color was good.

On the seventh day he developed transient episodes of cyanosis following feedings. A roentgenogram of the chest revealed cardiomegaly and partial atelectasis of the left upper lobe. An iodized oil U. S. P. (Lipiodol) swallow showed a dextroposed stomach. An electrocardiogram indicated a sinus rhythm with right ventricular hypertrophy and right axis deviation. The patient was digitalized and placed in a respirator. Pulse rate was 160 and respirations were 45 a minute. There

Fig. 1.—Multilobulated spleen of a 15-day-old infant.

developed marked dyspnea and cyanosis. Congestive heart failure and pulmonary edema ensued, and the infant died on Aug. 7, at the age of 15 days. The clinical diagnosis was "congenital heart disease with levocardia and situs inversus."

*Necropsy Findings.*—The body of the infant was 45 cm. long and weighed 2850 gm. There was moderate edema of the soft tissues. No externally visible anomalies were present. In the peritoneal cavity, the stomach, the pancreas, and the spleen were transposed into the right upper quadrant. The lower margin of the right lobe of the liver extended 2.0 cm. below the right costal margin. The liver had the usual configuration and weighed 120 gm. The splenic tissue weighed 6 gm. and consisted of 16 lobules (splenunculi), which were up to 1.2 cm. in diameter. These were held together by delicate bands of connective tissue

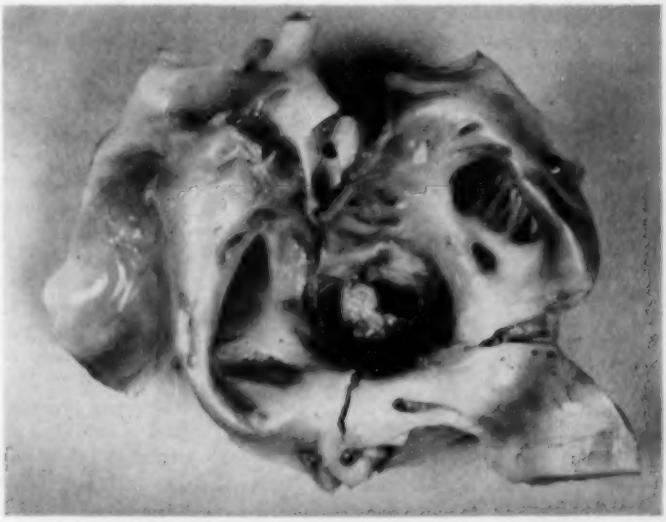


Fig. 2. — Congenital heart (cor triloculare biventriculare). The orifice shown is the only valve between the ventricles and a common atrium. Note the two atrial appendages.

(Fig. 1). There was inversion of the entire intestinal tract, and the cecum and the appendix were in a midposition between the left upper and the left lower quadrant.

The heart weighed 30 gm. There was considerable dilatation of the right ventricle. The four pulmonary veins, the superior vena cava, the inferior vena cava, and the coronary sinus emptied into a common atrium. Two distinct auricular appendages were present. In the floor of the common atrium there was a single atrioventricular orifice with four valve leaflets, two opening into the right ventricle and two into the left ventricle (Fig. 2). The pulmonary valve and artery were well formed. The ductus arteriosus was 0.2 cm. in diameter. The left coronary artery had a normal origin and distribution. The right coronary ostium was 1.5 cm. above the superior margin of the right cusp of the aortic valve; the artery had the usual distribution.

The lungs were well developed, with three lobes to the right lung and two to the left lung. The right lung weighed 40 gm.; the left weighed 30 gm. Focal areas of atelectasis were present throughout both lungs. Fluid exuded freely from the cut surfaces.

No anomalies were seen in other organs. Histologic examination of tissues yielded no additional findings.

*Anatomic Diagnoses.*—Congenital malformation of the heart (cor triloculare biventriculare); multilobulated spleen; situs inversus, involving the stomach, pancreas, spleen, and intestinal tract, and focal atelectasis and edema of the lungs.

## Comment

Ivemark noted that congenital absence of the spleen is usually associated with incomplete fusion of the atrioventricular cushions of the heart. Thus, his review of 67 cases collected from the world literature revealed 65 with various types of conotruncus anomalies (persistent truncus arteriosus, pulmonary atresia, transposition of the great vessels, cor biloculare, cor triloculare biatriatum, or combinations of these defects). This association is explained by the fact that the splenic primordium, an area of thickening in the dorsal mesogastrium, is first seen at the time when fusion of the atrioventricular cushions is taking place (8-10 mm. embryo, with ovulation age of 31-36 days). It has been postulated that the same factors which prevent the proper formation of the spleen also interfere with the usual fusion of the atrioventricular cushions.

In addition to the cardiac malformations, asplenia was associated with situs inversus of the stomach in 33 of 67 reported cases. Of the four cases of multiple spleens which Ivemark reported, three had dextroposed stomachs. Thus roentgenological examination of the stomach may be a useful aid in the diagnosis of this complex of developmental abnormalities. In general, these patients are not improved by surgery; in fact, several have died during or shortly after surgical intervention. At least 90% of infants with these anomalies do not survive the first year of life.

## Summary

Infants with multilobulated spleen frequently have an associated malformation of the heart in which there has been anomalous development of the atrioventricular cushions. Many of these infants also have a dextroposed stomach.

A case is reported in which a multilobulated spleen was associated with cor triloculare biventriculare. Situs inversus of the stomach, spleen, pancreas, and intestinal tract was also present.

Baylor University College of Medicine.

## SELECTED BIBLIOGRAPHY

Boggs, J. D., and Reed, W.: Congenital Absence of the Spleen, Quart. Bull. Northwestern Univ. M. School 27:289, 1953.

Bush, J. A., and Ainger, L. E.: Congenital Absence of the Spleen with Congenital Heart Disease, Pediatrics 15:93, 1955.

Ivemark, B. I.: Implications of Agenesis of the Spleen on the Pathogenesis of Cono-Truncus Anomalies in Childhood, Acta pediat. (Supp. 104) 44:1 (Nov.) 1955.

Miller, A. J.: Congenital Heart Disease with Partial Situs Inversus, Absence of Inferior Vena Cava, and Other Anomalies, Am. J. Path. 1:467, 1925.

Polhemus, D. W., and Schafer, W. B.: Congenital Absence of the Spleen; Syndrome with Atrioventricularis and Situs Inversus, Pediatrics 9:696, 1952.

Royer, B. F., and Wilson, J. D.: Incomplete Heterotaxy with Unusual Heart Malformations: Case Report, Arch. Pediat. 25:881, 1908.

# Lesions in Kidneys Removed for Unilateral Hematuria in Sickle-Cell Disease

F. K. MOSTOFI, M.D., Washington, D. C.; LIEUT. COL. COLIN F. VORDER BRUEGGE (MC), U. S. Army, and L. W. DIGGS, M.D., Washington, D. C.

Sickle-cell disease * as a cause of unilateral hematuria has attracted considerable attention since the report of Abel and Brown [1] in 1948. These authors reported bleeding from the left kidney of a Negro soldier, who had only slight anemia and no jaundice. Pyelograms disclosed a filling defect in the pelvis of the left kidney. A renal neoplasm was suspected, and nephrectomy was done. Pathologic examination revealed only generalized stasis of sickle cells, focal hemorrhage, and necrosis of the renal pelvis. Interest in hematuria as a manifestation of sickle-cell disease has been further stimulated by the reports of Goodwin, Alston, and Semans [6]; Ryan and Fuller [9]; Creecy, Varano, and Hurdle [4]; Harrison and Harrison [7]; Smith and Conley [10]; Lund, Cordonnier, and Forbes [8]; Arduino,[2] and Chapman et al.[3] Collectively, these authors have described 58 patients with hematuria associated with sickle-cell disease, including 16 patients whose hematuria began unilaterally and on whom nephrectomy was performed. The commonest indications for the removal of the kidney were the severity of the blood loss and the possibility of renal neoplasm.

The purpose of the present paper is to describe the pathologic changes in the kidneys of a group of patients with sickle-cell disease and unilateral hematuria.

## Selection of Material

This paper is based upon the study of 22 Negro patients with sickle-cell disease and unilateral hematuria, whose clinical records and surgical specimens were available in the files of the Armed Forces Institute of Pathology. In 21 cases the kidney had been removed; in 1 case a wedge biopsy had been performed. In 10 of these cases the diagnosis of sickling was established by clinical tests; sickled cells were unmistakable in tissue

Submitted for publication Sept. 27, 1956.

From the Armed Forces Institute of Pathology, Washington, D. C.

Present addresses: Armed Forces Institute of Pathology and Central Laboratory for Anatomical Pathology and Research, Veterans Administration, Washington, D. C. (Dr. Mostofi); Walter Reed Army Institute of Research, Washington, D. C. (Lieutenant Colonel Vorder Bruegge); University of Tennessee College of Medicine, Memphis (Dr. Diggs).

* Sickle-cell disease: A term used to include all categories of disorders characterized by the presence of hemoglobin S.

Sickle-cell phenomenon: The change of erythrocytes from discoid to bizarre and elongated "sickle" forms due to tactoid formation of reduced hemoglobin S within the erythrocytes.

Hemoglobin S: A genetically determined type of abnormal hemoglobin found in approximately 10% of Negroes, which has a characteristic electrophoretic mobility and a significantly decreased solubility associated with tactoid formation when the hemoglobin is in the deoxygenated (reduced) state.

Sickle-cell trait: (1) The hereditary condition in persons who are heterozygous for the sickling character, usually not associated with anemia, increased red cell destruction, and episodes of pain and fever, but compatible with an essentially normal life expectancy; (2) the inherited hemoglobin-S abnormality.

Sickle-cell anemia: A hereditary disease characterized by homozygosity for hemoglobin S and the presence of variable amounts of hemoglobin F in the absence of hemoglobin A (normal adult hemoglobin) and by the manifestations of severe chronic hemolytic anemia, recurrent febrile and painful "crises," obliterative vascular lesions, and reduced life expectancy.

Sickle-cell-hemoglobin-C disease: A hereditary syndrome characterized by the presence of hemoglobins S and C, and by chronic, usually low-grade, anemia of the hemolytic type and a clinical picture which is usually intermediate in severity between the sickle-cell trait and sickle-cell anemia.

sections, and certain characteristic pathologic changes were consistently observed in the kidneys removed surgically. The other 12 cases showed exactly the same clinical and pathologic picture: There was unequivocal sickling in tissue sections and no other demonstrable cause for hematuria. Even though in 2 of these patients the sickling test was said to be negative and in the other 10 the test either was not done or, if done, was not recorded, we believe that these 12 cases can be included with the 10 for which clinical evidence of sickling was available. In the cases with and those without positive clinical tests for sickling, the diagnosis of sickle-cell disease was confirmed or established by the finding of elongated, double or multipointed, crescent or spindle-shaped erythrocytes (sickle cells) in formalin-fixed kidney tissue (Fig. 1). Sickling of red blood cells varied in degree in different specimens and in different areas and blocks of the same tissue, but it was always unequivocal.

Excluded from this study were 24 other cases with similar histories: 10 because the red blood cells in tissue, although abnormal, did not show unequivocal sickling, and 14 because a coexisting disease process could not be ruled out as a contributing cause of hematuria, even though unmistakable sickle cells were seen in the excised kidney.

Classification of the cases of our series into well-defined categories, such as sickle-cell trait, sickle-cell anemia,* or sickle-cell-hemoglobin-C disease * was not possible, because reports on electrophoretic studies were available in only a few cases and some clinical records were lacking in essential details. Laboratory tests that might have clarified the true situation in many instances had not been performed, and our efforts to obtain further clinical verification while conducting the pathologic investigations have been unsuccessful. From the information available, interpreted in the light of our own experiences, we believe that the

Fig. 1.—Elongated, curved, and pointed (sickled) red blood cells in the kidney. AFIP Acc. 629356; × 860.

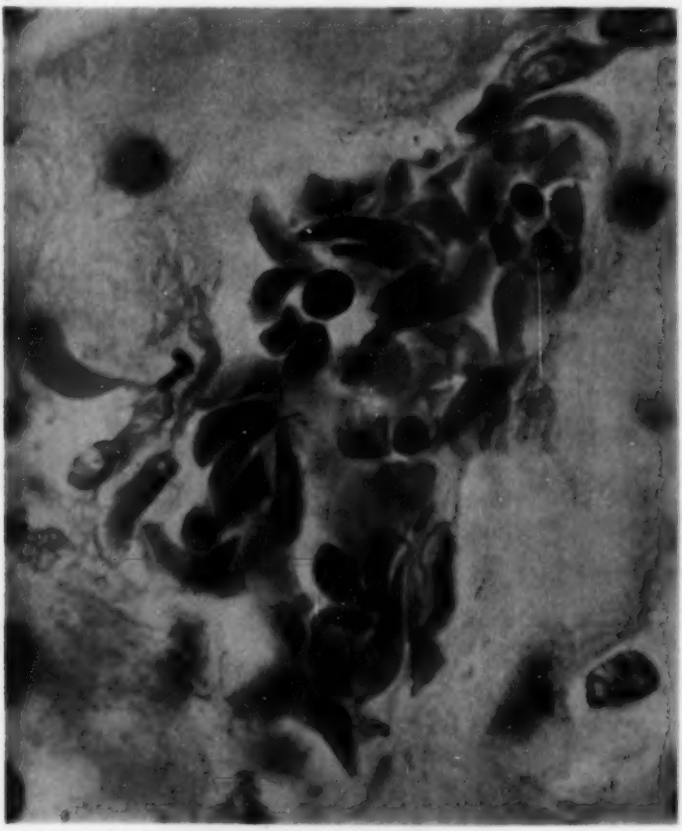


TABLE 1.—*Clinical Data on Twenty-Two Patients with Hematuria Associated with Sickle-Cell Disease* *

| Case No. | AFIP Acc. No. | Age and Sex | Duration of Bleeding on Admis. | Pain at Onset | Previous Hematuria | Summary of Clinical Record | Laboratory Examinations | | | Cystoscopy and Pyelograms | | Reason for Nephrectomy | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Sickling Test | Hemoglobin and/or RBC | Other (Exclusive of RBC in Every Urine Preoperatively) | Kidney Involved | Filling Defects and Other Findings | Neoplasm ? | Blood Loss ? | Other |
| 1 | 91686 | 25 M | 6 mo. | – | – | Bright red blood intermittently for 6 mo.; flank pain (on adm.), rheumatism, enuresis; frequency, urgency at 7, 12, & 14 yr. | P.O. 1 neg. | 85% 5,800,000 | Urine: Neg. for TB & casts; BUN normal | Lt | No abnormality | + | + | |
| 2 | 134332 | 26 M | 18 mo. | – | – | Continuous hematuria since "blow, lt flank" visual disturbances, 13 mo. | Several neg.; 1+ | .. | | Lt | Gross hematuria from lt kidney; no deformity of pelvis or calyces | + | | |
| 3 | †176626 | 19 M | 3 mo. | – | + | Intermittent hematuria, aggravated by exercise; urethral polyp resected 13 mo. after onset of hematuria; later, RBC 4,300,000; lt flank pain and tenderness; arthralgia, arms and knees; aspiration biopsy, 2 mo. before nephrectomy; no tumor cells | Severe sickling | 2,000,000<br>1,980,000<br>5,150,000 | Urine: Neg. for TB | Lt | Hematuria, lt. Three pyelograms: 1, Defect, lt renal pelvis<br>2:Normal<br>3:Incomplete filling defect, lt | | + | + |
| 4 | 180354 | 19 M | 2 yr. | | + | Intermittent gross hematuria for 2 yr. | No inf. | No inf. | Urine: tumor cells? | Lt | Blood from left ureteral orifice; otherwise neg. | + | | |
| 5 | 200535 | 30 M | 6 wk. | – | .. | Gross hematuria, painless; P.E. negative 3 yr. P.O. | No inf. | No inf. | Urine: Neg. for TB & neoplastic cells | Lt | Superior pole defect | + | | |
| 6 | 277900‡ | 23 M | 2 mo. | – | + | Hematuria, intermittent, 10 mo.; pyelograms disclosed defects on left, but bleeding was from rt kidney<br><br>After rt nephrectomy occasional bleeding from lt kidney for 4 ½ yr. | + | 10 to 14.5 gm.<br>3,500,000 | Urine: Neg. for TB; 24 hr. urobilinogen 2 mg. prothrombin time, 79% | Rt; then lt | 1:Normal<br>2:Extrinsic pressure, lt. ureteropelvic junction?<br>3:Nodules?; upper lt. ureter | + | + | |
| 7 | 298389 | 21 F | 10 days | – | – | Painless hematuria | + | 14 gm.<br>4,640,000 | Urine: Pos. for albumin; sp. gr. 1.006 | Lt | Superior pole, lt; Neoplasm(?) | + | | |
| 8 | 300341 | 19 M | 1 wk. | – | .. | Gross hematuria, painless<br><br>Temporary "iritis," several mo. P.O.; no hematuria | No inf. | .. | Urine: Many intact RBC | Lt | Questionable defect upper calyx, left | + | | |
| 9 | 309797 | 23 M | 1 day | + | + | Episodes of painless hematuria, 6 & 1 yr. previously; backache, pain in LLQ; no hematuria 2 yr. P.O. | + | 16 gm. | Many laboratory tests neg. | Lt | 1:Sl. dilated pelvis, lt<br>2:Same<br>3:Same, esp. lt upper calyx | | | |
| 10 | 461933 | 24 M | 2 wk | – | | Gross hematuria, onset with respiratory infection; cont. bleeding, no other symptoms; 6 preop transfusions; surgical shock, 2 transfusions; oliguria; death, 38th day, in uremia; no autopsy | + | 11 gm. | Hemoglobin 3 gm. few days after onset | Lt | 1:Phleboliths, incomplete filling, superior calyx, lt; defect superior calyx, rt<br>2:Same | | | |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 11 | 484442 | 22 M | 2 wk. | – | – | Hematuria after 1 mo. in Army; nocturia 2 times, no dysuria; mild preop. transfusion reaction; no hematuria or other complaints 3 yr. P.O. | No inf. | No inf. | Tests for intravascular hemolysis neg. | Lt | Filling defect, lt superior pole | + | + |
| 12 | 496294 | 29 F | 5 wk. | + | + | Hematuria for 3 wk. 1 yr. previously; bleeding, clots 2 days after jeep ride and 1 day after menstruation; colicky pain, rt flank, backache, nausea | Not done | 13 gm. | Hbg. in 2 wk. 6.5 gm.; NPN normal; urine: neg. TB, RBC; albumin 2 to 4+ | Rt | No significant abnormalities | | + |
| 13 | 503852 | 23 M | 3 mo. | + | + | Intermittent hematuria for 3 mo., also 6 yr. previously; left CVA pain, nausea, vomiting; 40 lb. wt. loss.; nephrectomy not done; wedge biopsy showed fetal lobulations | No Inf. | 14 gm. | Hematocrit 43% | Lt | Defect between mid and upper calyces, lt; 3 mo. later, 3 cm. mass? | + | |
| 14 | 523029 | 22 M | 6 days | – | | Hematuria, 5 episodes in 6 mo.; severe pain preoperatively; tenderness, lt flank; bleeding increased with exercise; at operation kidney normal except area of suspected carcinoma; no hematuria or other complaints 6 and 14 mo. P.O. | + | 16 gm. | 3 sickling tests neg. pre-op.; Pos. test P.O. | Lt | Slight pelvic deformity; question of aberrant vessel | + | |
| 15 | 540411 | 20 M | 3 days | + | | Intermittent hematuria 14 days; lt CVA pain; frequency | No Inf. | | No Inf. | Lt | Pelvic, lt, deformity | | |
| 16 | 569175 | 20 M | Few days | – | – | Continued hematuria, 5 mo.; mod. anemia; no effect from transfusions; ruptured appendix 3 mo. P.O.; low back pain, no hematuria 6 mo. later | + | 15.5 gm. 3,500,000 | | Lt | | | + |
| 17 | 629356 | 30 M | | + | + | Intermittent hematuria with clots for 6 mo.; pain, left flank | + | 14.5 gm. | Several sickling tests neg. before pos. test | Lt | Pelvic defect, lt | | + |
| 18 | 636652 | 23 M | 2 mo. | + | | Profuse hematuria, clots for 2 mo.; renal pain, 4 mo.; "low grade fever" | No Inf. | 6.7 gm. 2,420,000 | Urine: neg. for TB; alb-4+; atypical cells suggestive of Ca | Lt | Defect of upper calyces; appearance of TB (?) (Rt) Lt: clot (?) | + | + |
| 19 | 642841 | 29 M | Few days | + | + | Massive hematuria; similar episode 6 mo. previously; splenomegaly | + | 12.6 gm. 4,170,000 | RBC in 2 wk;-2,400,000; many target-cells | Lt | "Bulge" defect, anomaly (?) | | + |
| 20 | 663794 | 26 M | 2 mo. | | | Gross hematuria for 2 mo., always from rt | No inf. | No inf. | No inf. | Rt | None | | + |
| 21 | 665451 | 24 M | 3 days | – | + | Intermittent hematuria aggravated by exercise, 20 mo.; similar episode 10 yr. previously; on 2 previous admissions for hematuria blood and urine normal; on duty 3 mo. before this admission | + | RBC normal then 3,000,000 | 3 sickling tests neg. before pos. test; Hbg 6.2%, fetal type; retic. platelet counts-normal | Lt | | | + |
| 22 | 740538 | 18 M | | – | + | Hematuria 1 yr ago following football injury; sudden recurrence, painless total hematuria | + | | P.O. electrophoresis; A8 Hbg | Lt | Bleeding; constant filling defect, lt midcalyx | + | + |

* Table compiled by the Armed Forces Institute of Pathology, Walter Reed Army Medical Center.
† Reported as Case 1 by Goodwin et al.[6]
‡ Reported by Chapman et al.[5]
§ To be reported by Col. K. T. Artman (MC), U. S. Army Hospital, Ft. Knox, Ky.

basic condition was the sickle-cell trait,* because there was nothing in these patients' histories to suggest chronic anemia: no recurrent attacks of pain and fever, no jaundice, and no evidence of leg ulcers or enlargement of the heart or both. Red blood cells counts and hemoglobin values were normal or only slightly depressed.

## Clinical Data

Pertinent clinical information on each of the 22 patients is summarized in Table 1. Many of these patients were not anemic despite steady loss of blood as a result of hematuria. Those who were anemic had no signs of increased destruction or abnormal regeneration of red blood cells.

*History and Symptoms.*—Twenty of the patients were male and two were female. Their ages ranged from 18 to 30, with an average of 24 years. Gross hematuria was present in all patients. The onset was generally spontaneous and painless, but 7 of the 22 patients described pain or aching sensations in the back or the flank. In three male patients the pain shifted and localized in the lower abdominal quadrant on the affected side. Severe colicky pains with nausea and vomiting were recorded in the histories of only three patients. Urinary frequency, urgency, dysuria, and nocturia were rare during the initial episode. None of the patients had high fever, chills, evidence of pyuria, or pain referred to the perineum.

No other unequivocal causal factor to explain the hematuria and no associated illness could be determined in most of the cases. Mild upper respiratory infections preceded or accompanied onset of hematuria in 2 of the 22 patients. Three others believed the renal bleeding was associated with trauma to the flank or with lifting heavy objects. In several instances long marches or other unusual exertion increased the severity of bleeding or preceded a recurrence.

The duration of the gross hematuria ranged from days to months, the average being two months. Previous episodes of hematuria, sometimes over a period of several years, were described in 10 cases. Nine patients had suffered from intermittent hematuria for more than a year. Petechiae and purpuric manifestations had not been observed in any of our cases. No bleeding from mucous surfaces or evidence of telangiectasia had been recorded.

*Physical Examination.*—Most of the patients were well-developed and well-nourished young Negro men. None were acutely ill. Except for tenderness in the costovertebral angle over the affected kidney in six patients and splenomegaly in one (Case 19), the only significant symptom was hematuria.

The most important cystoscopic finding was unilateral renal bleeding in every patient. In 19 of the 22 cases blood was observed coming from the left ureter; it was "bright red" and said to be "spurting" and "pouring" from the ureteral orifice. No abnormalities were seen in the urethra or bladder. Blood clots in the bladder were noted occasionally. There was slight, if any, delay in the appearance of intravenous dyes in patients so tested.

*Laboratory Examination.*—Intravenous and retrograde pyelographic studies disclosed vague deformities or filling defects of the renal pelvis in 15 patients. Some reports described haziness of the outline of the infundibulum and lack of sharp definition in the outline of the calyces. Suggested roentgenological diagnoses were renal neoplasm, mild hydronephrosis, changes due to aberrant vessels or other sources of extrinsic pressure, congenital deformities, nonopaque stone in the pelvis, hemangioma, or blood clot.

In half the patients the filling defect was considered most suggestive of renal neoplasm. In several instances reexamination after a short interval showed that the defect was transitory or had changed in contour or size. In 8 of the 15 patients with reported defects, the location was in the upper pole. Roentgenograms of the chest and skeleton were made on several patients, but no significant abnormalities were noted.

On admission, the hemoglobin values were normal or only slightly decreased in 9 of the 22 patients. In five patients whose hematuria was extreme, hypochromic microcytic anemia developed rapidly, requiring multiple transfusions. A slight rise in reticulocytes and the occasional presence of nucleated red blood cells were typical of changes that might follow blood loss from any cause. The leukocyte counts were usually normal, as were tests for bleeding time, coagulation time, capillary fragility, clot retraction, and prothrombin levels. Positive clinical tests for sickling were reported, either before or after operation, in 10 patients; in 2 the test was recorded as negative; in 10 no information concerning the test could be obtained. The desirability of repeated tests is illustrated by three cases in which a number of negative sickling tests preceded the positive one.

Albumin and red blood cells were found in the urine. Clots were fairly common, but casts were infrequent. Renal function tests, including specific gravity, were normal. Cells suggestive of carcinoma were seen in the sediment of urine from two patients. Urine from six of the patients on whom the test was performed was negative for acid-fast organisms on multiple examination by varied techniques. Routine urine cultures were usually negative, but in a few cases various organisms were grown.

*Clinical Course and Therapy.*—Little or no beneficial effect was derived from various methods of therapy, including lavage of the kidney pelvis with 0.5% solution of silver nitrate, x-ray therapy, and administration of vitamins C and K, or vasoconstrictor and antihemorrhagic drugs. Transfusions and bed rest improved the general condition of anemic patients but did not affect the hematuria. Renal neoplasm, tuberculosis, pelvic stone, or pelvic ulcer was the clinical diagnosis that led to nephrectomy. Seven patients bled so profusely that nephrectomy was resorted to "as a life-saving measure to prevent exsanguination."

Nephrectomy was performed without surgical complications, and convalescence was uneventful in all but one patient (Case 10), who went into shock during the operation. He had received six preoperative whole-blood transfusions, and two additional transfusions were given during the operation. He became oliguric and died in uremia 38 days after nephrectomy. Autopsy was not performed.

Removal of the affected kidney resulted in cessation of the hematuria in every case. During postoperative hospitalization, an immediate upward trend in erythrocyte values usually was evident. Most of the patients returned to military duty or their usual occupations. Further data are available in only eight of these cases. Periodic follow-up examinations disclosed no recurrence of hematuria in two patients observed for more than three years postoperatively (Cases 5 and 11), one patient for two years (Case 9), one for approximately one year (Case 14), and two for several months after operation (Cases 8 and 16). One airman recovered from a ruptured appendix three months after nephrectomy, and hematuria was not observed during the second hospitalization. Six months later he complained of low back pain and had tenderness in the costovertebral angle, but no hematuria (Case 16). Intermittent hematuria was reported during a follow-up period of more than four years after nephrectomy in one patient (Case 6). Renal bleeding was accompanied by low back pain, tenderness in the costovertebral angle, and painful priapism; but his general health remained good and he was only slightly anemic.

## Pathologic Observations

*Gross Examination.*†—The kidneys were somewhat swollen; the capsules stripped with ease, and the dark-red surfaces were smooth. Multiple sections failed to reveal evidence of tumor, tuberculosis, or other

† None of the kidneys were available to us for gross examination, and these descriptions are based on the recorded observations of the contributor.

TABLE 2.—*Histopathologic Data on Twenty-Two Patients with Hematuria Associated with Sickle-Cell Disease* *

| Case No. | AFIP Acc. No. | Cortex | | | | | Medulla | | | | | | | Papillae | | | | | | | Pelvic Mucosa | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Glomerular Stasis | RBC in Capsule & Proximal Tubules | Tubular Stasis | Pigment in Tubules | Casts | Stasis | Peritubular Hemorrhage | Tubular Hemorrhage | Edematous Areas | Fibrosis | Pigment in Tubules or Parenchyma | Casts | Stasis | Perivascular Hemorrhage | Epithelial Degeneration | Epithelial Proliferation | Necrosis | Fibrosis | Edematous Areas | Stasis | Hemorrhage | Epithelial Denudation |
| 1 | 91686 | + | − | + | − | − | + | − | − | + | + | + | − | + | + | + | | − | − | + | + | + | + |
| 2 | 134332 | + | + | + | − | Col. | + | − | − | + | + | + | − | + | + | + | | − | + | + | | + | + |
| 3 | 176826 | + | − | + | − | Hem. | + | − | − | + | + | + | − | + | − | + | | − | + | + | | + | + |
| 4 | 180384 | + | − | + | + | RBC | + | − | + | + | + | + | − | + | − | + | − | − | + | + | | | |
| 5 | 200535 | + | − | + | − | − | + | − | + | + | + | + | − | + | − | + | | − | + | + | + | + | + |
| 6 | 277909 | + | − | + | + | Col. | + | − | + | + | + | + | − | + | − | + | | − | + | + | | | |
| 7 | 298389 | + | − | + | + | Col. | + | − | + | + | + | + | − | + | − | + | + | − | + | + | | | |
| 8 | 300341 | + | + | + | + | − | + | − | + | + | + | + | − | + | − | + | | − | + | + | + | + | + |
| 9 | 309707 | + | + | + | + | − | + | − | + | + | + | + | − | + | − | + | | − | + | + | | | |
| 10 | 481933 | + | − | + | + | − | + | − | + | + | + | + | − | + | − | + | + | − | + | + | | | |
| 11 | 484442 | + | − | + | + | − | + | + | + | + | + | + | − | + | − | + | + | − | + | + | + | + | + |
| 12 | 496294 | + | + | + | + | RBC | + | − | + | + | + | + | − | + | − | + | + | − | − | + | | | |
| 13 | 503852 | + | − | + | − | − | + | − | − | + | + | + | − | | | | | | | | | | |
| 14 | 532029 | + | − | + | + | − | + | − | + | + | + | + | − | + | | + | + | + | | + | + | + | |
| 15 | 546411 | + | − | + | + | | + | − | + | + | + | + | − | | | | | | | | | | |
| 16 | 569175 | + | + | + | + | | + | − | + | + | + | + | − | RBC | − | − | + | − | − | + | | | |
| 17 | 629356 | + | + | + | + | RBC | + | − | + | + | + | + | − | Hem- | + | + | + | + | + | + | + | + | + | |
| 18 | 638852 | + | − | + | + | | + | − | + | + | + | + | − | | | | | | | | | | |
| 19 | 642841 | + | | + | + | | + | − | + | + | + | + | − | RBC | + | + | + | + | + | + | | | |
| 20 | 653794 | + | | + | + | | + | − | + | + | + | + | − | | + | | | | | | | | |
| 21 | 665451 | + | | | + | Col. | | | | + | + | + | − | | + | + | | + | + | + | + | + | |
| 22 | 740338 | + | + | + | − | Col. | + | + | + | + | + | + | − | − | + | + | + | + | − | − | + | + | + | + |

* Table compiled by the Armed Forces Institute of Pathology, Walter Reed Army Medical Center.

lesions suspected from the clinical evidence. Four kidneys (Cases 6, 8, 10, and 20) were described as normal. In one case where only wedge biopsy was performed (Case 13) the tissue "showed small tan punctate pigmented areas." In one case (Case 16) "a thin hemorrhagic zone of injection" was observed "around the outer 0.2 cm. of cortex." Congestion and focal hemorrhages were limited to the medulla in 2 cases (Cases 5 and 18) and to the cortex in one case (Case 13) and were described in both cortex and medulla in six cases (Cases 15, 16, 17, 19, 21, and 22). In one case (Case 2) "several irregularly contoured but circumscribed pallid areas, up to 1-0.5 cm.," were noted in the cortex. Congestion and hemorrhages in the renal pelvis were observed in the 16 cases in which the pelvis was described, with papillary necrosis an added feature in 3 of these cases (Cases 11, 17, and 21). Clots in the pelvis were described in two cases (Cases 1 and 22).

Fig. 2.—Severe medullary congestion. AFIP Acc. 740338; × 15.

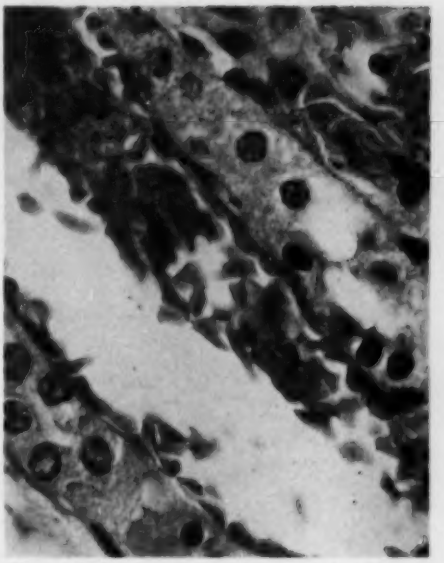


Fig. 3.—Higher magnification, showing abnormal appearance of the red cells. Sickled cells, pointed cells, and elongated cells are numerous. AFIP Acc. 740338; × 680.

The disappointing absence of any significant gross alterations in most of these kidneys emphasizes the fact that the lesions are inconspicuous and minute and may be easily missed.

*Microscopic Examination.* — The fundamental and most characteristic feature of these kidneys was the invariable presence of sickled erythrocytes (Figs. 1, 3). Details of the other pathologic changes in each case are listed in Table 2. The blood vessels showed patchy, but severe, stasis. Widely dilated capillaries were crammed with sickled red blood cells, but no thrombi were identified. The intertubular capillaries often displayed swelling or proliferation of endothelium with the cells protruding into the vascular lumen in a manner simulating epithelial cells.

In the glomeruli, puddling of blood was noted in the capillary loops, with some segments of the capillaries packed with sickled erythrocytes and others free of congestion. No thickening of the basement membrane, increased cellularity, adhesions, intracapil-

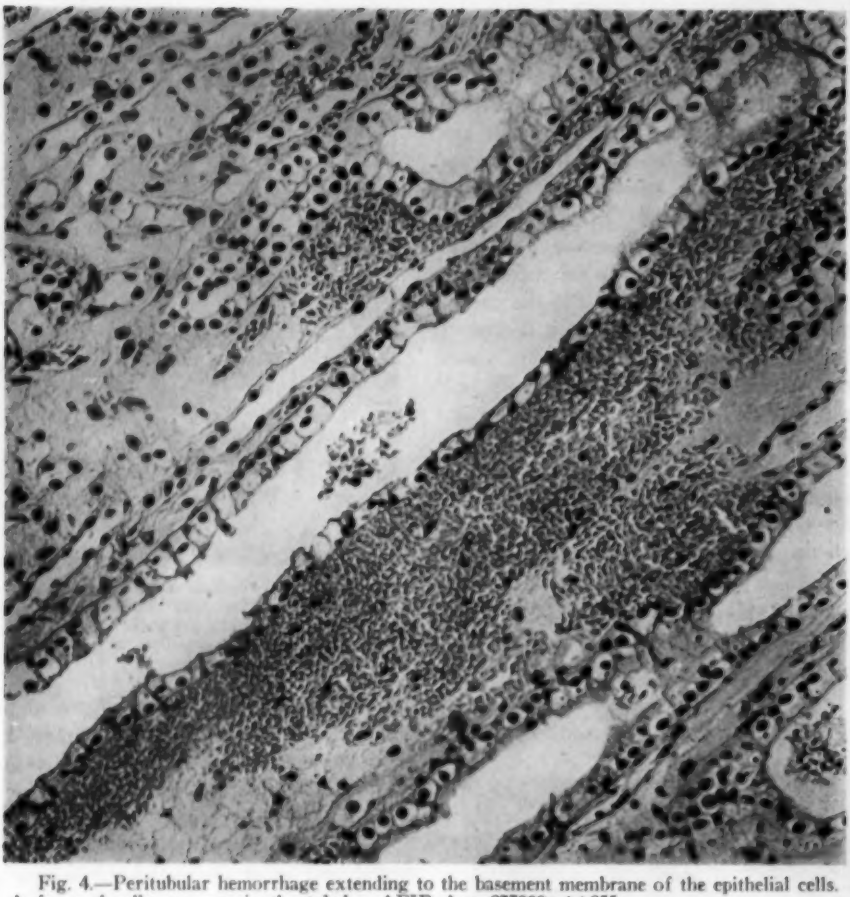


Fig. 4.—Peritubular hemorrhage extending to the basement membrane of the epithelial cells. A few red cells are seen in the tubules. AFIP Acc. 277909; ×255.

lary fibrin thrombi, or periglomerular infiltrates were seen.

The most striking change consisted of severe stasis in peritubular capillaries of both the cortex and the medulla, most marked in the medulla (Figs. 2, 3). In cross section, the packed sickled erythrocytes often had the appearance of a collar around the tubules. Individual, well-preserved erythrocytes were sometimes superimposed on or intermingled with degenerating epithelial cells.

Extravasation of blood into peritubular areas or into the tubules, with separation of tubular epithelium from the basement membrane and desquamation of the epithelial cells, apparently was a later development. Red blood cells were seen in the glomerular spaces or the adjacent convoluted tubules of the cortex or both, in 10 cases, always in association with medullary congestion and hemorrhage. In these 10 cases, and in 6 others, extravasation of blood was observed mainly in relation to the collecting tubules. In small segments of the tubules, foci of degeneration were sharply demarcated from minute regions of regeneration or from denuded areas. Although clinical data indicated frequent episodes of hematuria, blood or blood-derivative casts were found in tissue sections in only five instances; in seven others occasional casts were composed of

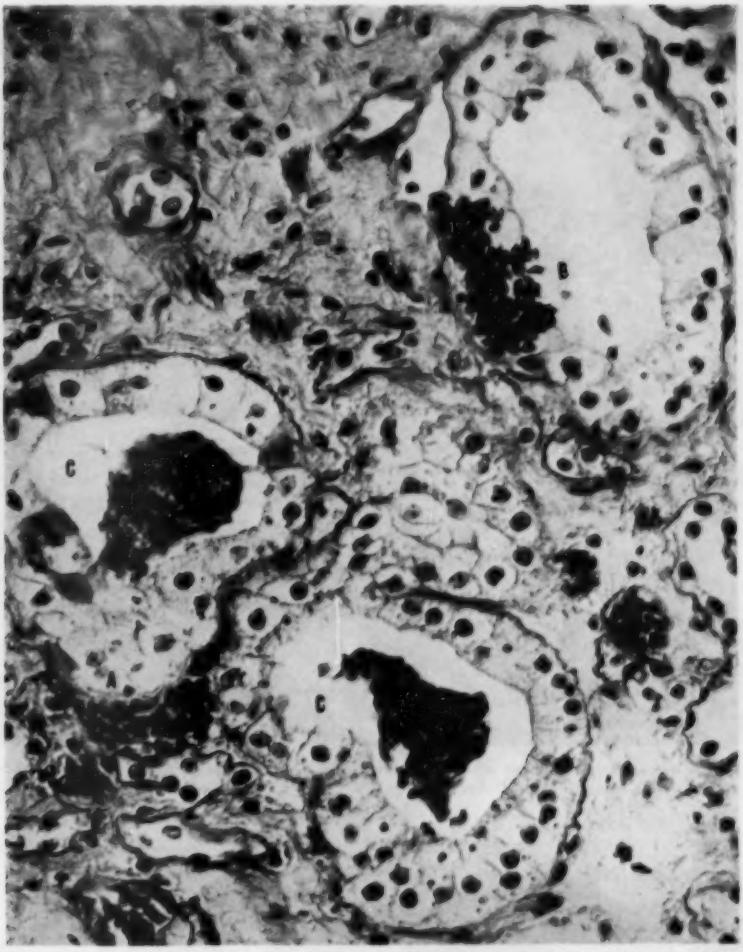



Fig. 5.—Cross section of three tubules. Peritubular hemorrhage at *A*; intraepithelial hemorrhage, *B*, and intratubular hemorrhage, *C*. Many of the epithelial cells contain cytoplasmic inclusions. AFIP Acc. 629356; reduced to about 78% of mag. × 600.

cellular debris and a lightly stained, eosinophilic, proteinous colloid substance.

Older lesions were characterized by partial or complete loss of the epithelium in the segment involved and by residual iron pigment in the adjacent epithelial cells or the stroma, suggesting resorption of hemorrhage. At this stage the small empty spaces, sometimes partly lined by remnants of epithelial cells or entirely lined by fibrous tissue (Fig. 6), were construed as erstwhile tubules. The edematous supporting connective tissue in these areas was infiltrated with scattered lymphocytes, monocytes, and hemosiderin-laden macrophages. Such areas were found in all instances. Iron pigment was also seen as dust-like granules in the epithelial cells of some adjacent tubules in 17 cases. Other epithelial cells occasionally contained intracytoplasmic material that suggested degenerating red blood cells.

In addition to the small foci of recent or old hemorrhage, or both, and the edematous areas already described, small stellate or

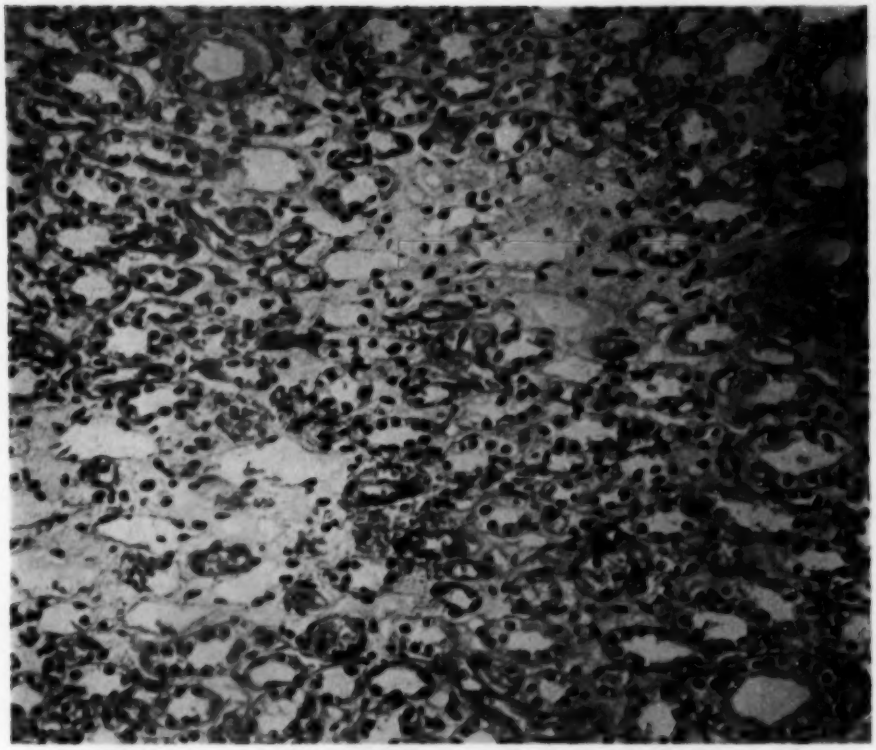


Fig. 6.—Two areas of tubular epithelial loss in the medulla. Some of the spaces probably represent erstwhile tubules. AFIP Acc. 277909; × 215.

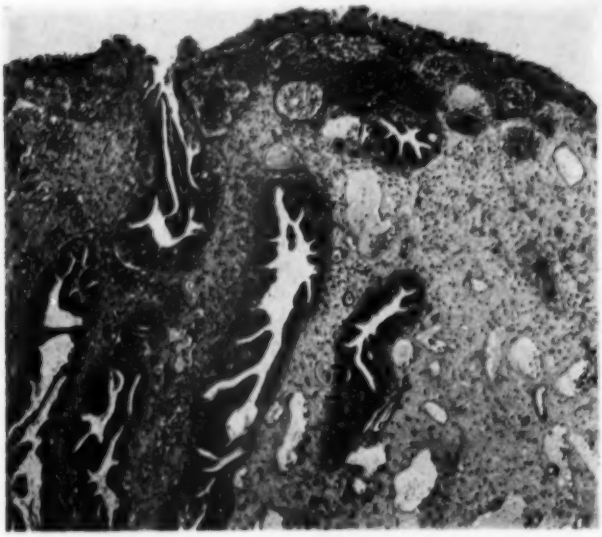


Fig. 7.—Hemorrhage in the tip of a papilla. Edema and fibrosis of the stroma and epithelial proliferation in the distal portion of collecting tubules indicate previous episodes of damage and repair. AFIP Acc. 298389; × 62.

Fig. 8.—Papillary necrosis. There is hemorrhage in the remainder of the papilla. AFIP Acc. 298389; × 55.

longitudinal areas of dense fibrous tissue, with or without hyalinization, were seen in the renal medulla in 20 cases. Scattered lymphocytes and monocytes and deposits of intracellular and extracellular iron pigment also were present in these areas. It is probable that these small scarred areas represented the terminal stages of lesions resulting from repeated episodes of renal hemorrhage (and hematuria). Similar foci of hemorrhage and progressive stages of fibrosis were encountered in the calyceal stroma of six of the cases.

Focal involvement of the renal papillae was another important manifestation of the disease (Table 2; Figs. 7, 8, 9, and 10). The papillary lesions included (1) marked vascular engorgement, which was seen in 14 cases; (2) edema of the subepithelial tissue, which imparted a myxomatous appearance to the stroma of the papillae in 19 cases; (3) subepithelial hemorrhage in 10; (4) degenerative changes of the papillae in 7, with frank necrosis in 2, and (5) loss of epithelium in areas of degeneration and

Fig. 9.—In the tip of another papilla than that in Figure 8, fresh hemorrhage is seen at *A*, ulceration at *B*, and myxomatous change at *C*. The details are shown in Figure 10. AFIP Acc. 298389; × 19.

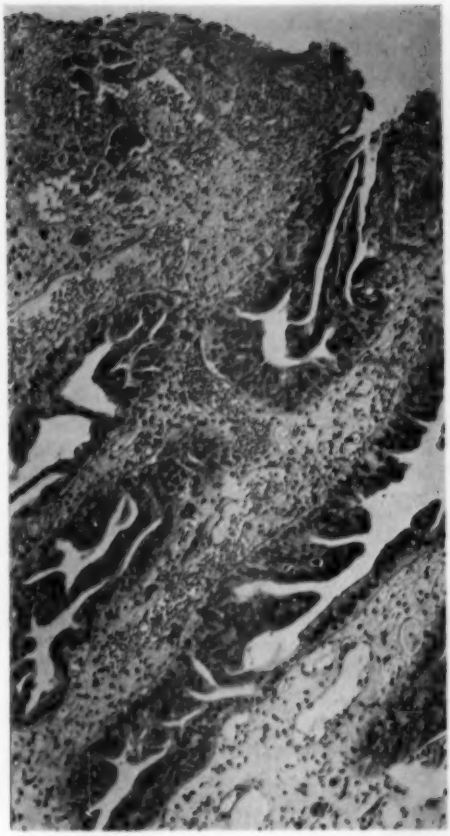


Fig. 10.—Section from the base of papillary necrosis observed in *A*, Fig. 9, showing hemorrhage and ulceration of the mucosa. Note the proliferative activity of the distal collecting tubules, indicating previous episodes of damage and repair. AFIP Acc. 298389; reduced to about 82% of mag. × 120.

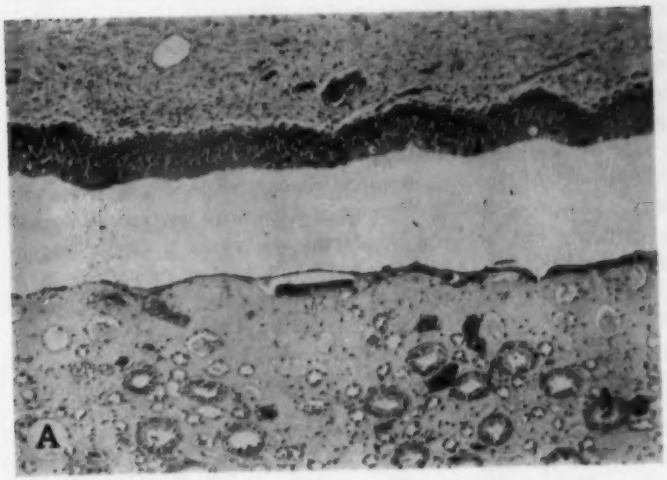
desquamation of the dead tissue in necrotic areas, exposing the base. Proliferation of the epithelium of the collecting tubules (Figs. 7 and 10) was seen in 12 cases. Stasis and subepithelial hemorrhages elsewhere in the pelvis were noted in 13 instances, in 8 of which the epithelium was lost. Regeneration or proliferative activity of the pelvic mucosa, or both, were seen in 14 instances (Fig. 11). Epithelial vacuolation was present in 10 cases, and in 3 of these the epithelial cells contained intracytoplasmic material that resembled degenerating red blood cells. Subepithelial hemorrhage without necrosis was also seen in the ureter in four cases.

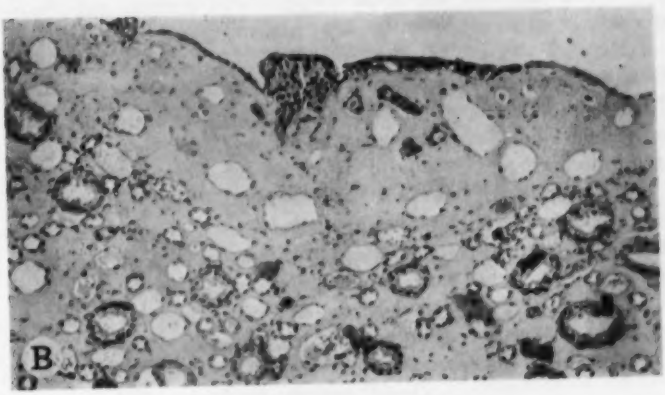
## Comment

In 22 cases of unilateral hematuria the diagnosis of sickle-cell disease was based on the observation of sickling of erythrocytes in formalin-fixed tissue, together with characteristic clinical manifestations. In 10 cases the diagnosis was possible from clinical evidence alone, for the sickle-cell test was positive. In the other 12 cases the clinical manifestations and pathologic changes were identical, but the sickle-cell test either had not been performed or was negative. In all cases the only demonstrable cause of hematuria was the abnormality of the red blood cells.

The question may well be asked as to whether a reliable diagnosis of sickle-cell disease can be based entirely on the appearance of erythrocytes in formalin-fixed tissue, as formalin fixation may also alter the appearance of the red blood cells. Our experience has convinced us that true sickling can be determined histologically. While it is true that formalin fixation can cause distortion of red blood cells, as a rule it preserves them in whatever shape they happen to be. When it does distort normal cells, they are irregularly shrunken, with rounded margins and blunt projections. They can be distinguished readily from true sickle-cells, with their sharp-pointed projections and crescent shapes that persist in formalin-fixed tissue.

In our experience, formalin fixation does not make sickling more pronounced. If anything, it tends to make sickling less conspicuous, for the changes, as in normal erythrocytes in tissue, consist of shrinkage and rounding of outline. Even in tissues from cases of sickle-cell anemia with massive sickling in peripheral blood samples, the effect of formalin fixation may be such as to obliterate evidence of sickling. Another factor which causes distortion of red blood cells is the prolonged exposure of unfixed tissue to air, but, again, it obscures



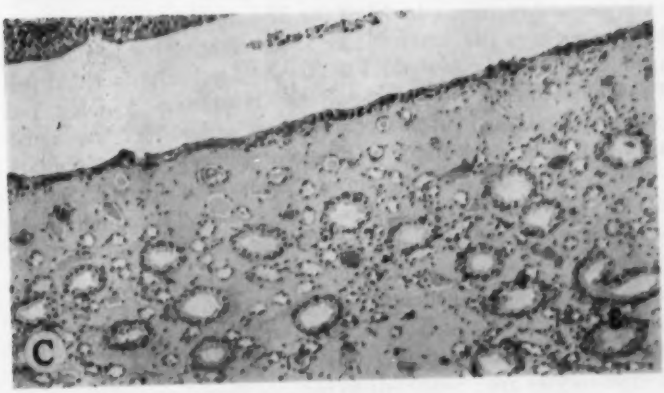




Fig. 11.—*A*, *B*, *C*, three stages in the regeneration of pelvic mucosa. *A*, the regenerating epithelium is represented by a few basal cells. In contrast to this, the opposing side is lined by normal transitional epithelium. *B*, the healing process is more advanced, almost covering the lamina propria. *C*, the mucosa is completely covered. Note the myxomatous appearance of the supporting stroma and absence of any inflammatory reaction. AFIP Acc. 663794; × 80.

rather than intensifies the morphologic features. It is our belief that unless tissue is subjected to undue exposure to air before formalin fixation, the red blood cells, including the sickled ones, will be preserved in essentially unchanged form, and that any distortion will be on the side of decreased rather than increased prominence of the morphologic characteristics. In properly fixed tissue true sickling certainly can be distinguished from formalin distortion.

Furthermore, the sickling of sickle-cell trait can be differentiated from that of sickle-cell anemia or hemoglobin-S-C disease. In the former, elongation of the red cells is not as marked as in the latter and the altered cells are observed focally rather than massively. The cases selected for this study were those in which the red blood cells were unequivocally sickled; but, in contrast to sickle-cell anemia, the sickling was not massive.

The basic lesion in these kidneys consisted of stasis of sickled red cells in small vessels, principally in the medulla and the papillae, with resultant focal hemorrhages in the interstitium, in the tubules, or in the pelvis. Fresh hemorrhages were frequent in the lumens of the renal tubules during acute hemorrhagic episodes, but heme casts or any other evidence of intravascular hemolysis, lower nephron nephrosis, or glomerular damage was conspicuously absent.

We believe that renal hemorrhage in patients with sickle-cell trait is secondary to hypoxia and resembles the hemorrhages in patients with sickle-cell anemia. The hemorrhagic manifestations of sickle-cell anemia have been attributed to vascular occlusive phenomena.[4] The abnormal hemoglobin in patients with sickle-cell trait is believed to crystallize on deoxygenation. Crystallization of hemoglobin in these cells produces relatively rigid, brittle, elongated, and spicular forms, which pass through the capillaries with more difficulty than normal cells. Increased viscosity of the blood in which erythrocytes are sickled results in stasis, followed by ischemia and/or infarction and/or perivascular edema, exudation, and hemorrhage. The end-stage is necrosis, fibrosis, and atrophy. The paucity of hemoglobin casts at the sites of hemorrhage in the kidney medulla is explained by the fact that the glomerulus above the lesion was actively functioning and the urine continually washed red blood cells and pigment away. Degenerative changes in the tips of papillae were followed by necrosis and slough of the tip, leaving relatively clean and vascularized proximal kidney tissue.

Hematuria is commoner in patients with sickle-cell anemia than in those with sickle-cell trait. The urologist, however, sees few patients with sickle-cell anemia for two reasons: (1) Their prominent symptoms are those of hemolytic anemia, and consequently these patients come under the care of the internist; (2) the incidence of sickle-cell anemia is much lower than that of sickle-cell trait, in a ratio of 1:40. Hematuria in patients with sickle-cell trait, many of whom are otherwise asymptomatic, is such an alarming initial manifestation that surgical intervention might seem to be indicated. In patients with sickle-cell anemia, on the other hand, hematuria is incidental to the well-recognized and dramatic symptoms of the disease. Radical treatment (nephrectomy) is rarely suggested in sickle-cell anemia, for hematuria is accepted as one of the manifestations of the disease.

In 19 of the 22 patients the left side was involved; in 3, the right. This predilection for the left may be due to the fact that the tributary of the renal vein draining the upper pole passes under the left renal artery as this vein joins the other tributaries to form the main renal vein, again favoring stasis of blood. The pathogenesis of the more frequent medullary involvement and the papillary necrosis observed in these patients remains obscure.

It is of interest that in none of these patients was there clinical evidence of nephrosis to indicate thrombosis of the renal

vein. Furthermore, none of the microscopic sections showed the fibrin thrombi in the glomerular capillaries that are observed in sickle-cell anemia. There is, therefore, no indication for a postulation of thrombosis as the underlying mechanism for either stasis or hemorrhage.

Our studies do not permit any statement concerning the trigger mechanism that sets off the chain of events leading to the renal lesions and the clinical observation of hematuria.

The treatment of these patients offers a challenge to the urologist. He must consider sickle-cell trait in every healthy young Negro patient with unilateral hematuria and must avoid nephrectomy unless there is a definite indication other than simple hematuria for such a radical operation. Not only is there no guarantee that hematuria will not recur from the opposite kidney, but such an eventuality is a distinct possibility.

## Summary

Twenty-two young Negro patients who were treated for severe hematuria with nephrectomy (21 patients) or renal biopsy (1 patient) were shown to have sickling of red blood cells in the resected kidney. The changes in the kidney consisted of severe congestion, focal medullary or papillary hemorrhages, necrosis, repair, and regeneration. In healthy young Negroes, if other causes for hematuria can be ruled out, it is advisable to avoid nephrectomy.

Armed Forces Institute of Pathology, Walter Reed Army Medical Center, 6825 16th St. N. W. (25).

## REFERENCES

1. Abel, M. S., and Brown, C. R.: Sickle Cell Disease with Severe Hematuria Simulating Renal Neoplasm, J. A. M. A. 136:624-625, 1948.

2. Arduino, L. J.: Urologic Complications of Sickle Cell Disease, Am. Surgeon 20:1213-1218, 1954.

3. Chapman, A. Z.; Reeder, P. S.; Friedman, I. A., and Baker, L. A.: Gross Hematuria in Sickle Cell Trait and Sickle Cell Hemoglobin-C Disease, Am. J. Med. 19:773-782, 1955.

4. Creecy, A. A.; Varano, N. R., and Hurdle, T. G.: Hematuria as a Manifestation of Sickle Cell Disease, Virginia M. Month. 78:642-646, 1951.

5. Diggs, L. W., and Vorder Bruegge, C. F.: Vascular Occlusive Mechanisms in Sickle Cell Disease, J. Nat. M. A. 46:46-49, 1954.

6. Goodwin, W. E.; Alston, E. F., and Semans, J. H.: Hematuria and Sickle Cell Disease: Unexplained, Gross Unilateral, Renal Hematuria in Negroes, Coincident with the Blood Sickling Trait, J. Urol. 63:79-96, 1950.

7. Harrison, F. G., and Harrison, F. G., Jr.: Hematuria with Sickle Cell Disease, J. Urol. 68:943-949, 1952.

8. Lund, H. G.; Cordonnier, J. J., and Forbes, K. A.: Gross Hematuria in Sickle Cell Disease, J. Urol. 71:151-158, 1954.

9. Ryan, J. E., and Fuller, R. H.: Hemorrhagic Manifestations of Sickle Cell Disease, U. S. Armed Forces M. J. 2:623-633, 1951.

10. Smith, E. W., and Conley, C. L.: Clinical Features of the Genetic Variants of Sickle Cell Disease, Bull. Johns Hopkins Hosp. 94:289-318, 1954.

# Effects of Colchicine and Serratia Marcescens Polysaccharide on Protoplasmic Viscosity

## *I. Relationship of Protoplasmic Viscosity to Cell Division of Ehrlich Ascites Tumor Cells*

EDWIN T. NISHIMURA, M.D., and JOSEPH H. BAUM, B.S., Chicago

The inhibition of cell division induced by decreasing the protoplasmic viscosity of certain nonmammalian cells has been investigated extensively by Heilbrunn and collaborators.[1-3] It is generally conceded by the cell physiologists that the successful completion of mitotic division is, in part, dependent upon the development and the activity of normal spindle fibers. According to the colloidal theory of cell division, the mitotic spindle is a solid structure that arises from the fluid protoplasm as a result of mitotic gelation.[4,5] Observations of the changes in the protoplasmic viscosity during mitosis of the eggs of a sea urchin (Arbacia), mollusk (Cumingia), and sea annelid (Chaetopterus) show a sharp rise in viscosity prior to the appearance of the mitotic spindle.[6-8] This increase in the viscosity is believed to be dependent upon the gelation of the protoplasmic colloid. It is therefore conceivable that the prevention of protoplasmic gelation would disturb the spindle formation and cause the cell to become arrested during mitosis. The phenomena of C-mitosis induction and the arrest of mitosis at metaphase in plant and animal cells treated with colchicine are believed by some to be related to a decrease in the protoplasmic viscosity of the cells.[9-13] Similarly, the inhibition of division in certain marine eggs treated with Serratia marcescens polysaccharide, as well as polysaccharides derived from animal tissues, is thought to be a result of alterations in the viscosity of the cells.[1-3]

In this investigation, an attempt was made to correlate the fall in the protoplasmic viscosity of the Ehrlich ascites tumor cells with the mitotic indexes during a finite period in the active growth phase of the tumor while the cells were exposed to the action of colchicine or S. marcescens polysaccharide.

### Materials and Methods

The Ehrlich ascites tumor* was selected for this study because the isolated tumor cells were considered well adapted for the estimation of (1) the relative protoplasmic viscosity,[14] (2) the cell multiplication rate during the growth phase of the tumor,[15] and (3) the mitotic indexes[16,17] at varying intervals of growth.

The tumor was continuously maintained in male Strong A mice, 3 to 6 months of age, by successive intraperitoneal transplantations at weekly intervals, using a 0.2 ml. aliquot of the ascitic-fluid suspension from the donor for each mouse. The mice were in air-conditioned quarters and were maintained on unrestricted intake of water and Rockland Mouse Diet.†

All in vitro tests were performed on aspirated ascitic-fluid suspensions after 7 to 10 days' growth of the tumor. Each tumor-bearing mouse was killed by cervical dislocation, and the suspension from the animal constituted a single test sample.

In vivo tests were conducted on mice with tumor growths of 7 to 14 days. In these tests, control samples were aseptically aspirated through sterile

Submitted for publication Oct. 24, 1956.

From the Department of Pathology, Northwestern University Medical School.

This investigation was aided by a grant (CP-76) from the American Cancer Society and by research grants (C-1005 and C-1750) from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

* The original tumor was supplied by Dr. Robert Schrek, of Veterans Administration Hospital, Hines, Ill., in November, 1953.

† Arcady Farms Milling Company.

22-gauge hypodermic needles with tuberculin syringes. The test materials were then administered intraperitoneally, and subsequent tumor samples were aspirated from the abdominal cavity at various time intervals. Polar-cell base counts of uncentrifuged ascitic-fluid suspensions were always rechecked in those animals which required sampling of tumor after intervals of five hours or more following the injection of the test agent. This check was necessary because in a few instances it was observed that the percentage of polar cells in the uncentrifuged suspensions diminished after five hours following the administration of some test agents.

The determination of the relative viscosity value of the tumor cells was made by the technique previously reported by Nishimura, DiPaolo, and Hill.[14] In the present investigation a refrigerated International Centrifuge, Model PR-2,‡ with a high-speed head, was used to centrifuge the tumor-cell suspensions (9900 to 10,000×g for 30 minutes at 0 C). The polar cells§ were enumerated as previously described,[14] and the relative viscosity value (VV) was determined by comparing the percentage of polar cells in the aliquot of untreated and uncentrifuged ascitic-fluid suspension (base count of polar cells) with the percentage of polar cells in the aliquot of the treated and centrifuged sample:

$$VV = \frac{\%\text{ polar cells, untreated and uncentrifuged}}{\%\text{ polar cells, treated and centrifuged}}$$

The base count of polar cells was always made for each tumor suspension. Under the conditions of this test, the mean viscosity value (MVV) of untreated and centrifuged control tumor-cell suspensions for 30 mice was 0.96, with a standard error of ± 0.01.

## Experiments and Results

1. *Substances Inducing Reduction in Protoplasmic Viscosity of Tumor Cells.*—(*a*) In Vitro Tests: Since certain polysaccharides and the alkaloid colchicine inhibit mitotic division of cells, experiments were designed to test the effects of S. marcescens polysaccharide (MP5LL4),‖ a polysaccharide derived from Pseudomonas aeruginosa (Piromen ¶), and colchicine U. S. P. on the protoplasmic viscosity of Ehrlich ascites tumor cells. MP5LL4 polysaccharide and Piromen were prepared in a concentration to contain 100γ in 1.0 ml. of 0.85% NaCl solution.# The colchicine solution was prepared in concentration of 0.04γ/ml. in 0.85% NaCl.

After transferring the polysaccharides and colchicine in the amounts shown in Table 1 into plastic centrifuge tubes, 1.0 ml. of the ascitic-fluid suspension was added to each test substance. The mixture was agitated, covered with Parafilm, and allowed to stand for 15 to 30 minutes in the refrigerator at 15 C. After this period of exposure the standard centrifugation procedure was followed and the viscosity values determined, as previously outlined.

The results of these tests are summarized in Table 1. Isotonic saline, used as a vehicle for the preparation of the polysaccharides and colchicine solutions, has by itself no effect on the protoplasmic viscosity of the tumor cells. A significant depression of mean viscosity values is produced by MP5LL4 polysaccharide, Piromen, and colchicine at the range of concentrations used in this study.

In another in vitro test, the mean viscosity values were determined with three different concentrations of MP5LL4 polysaccharide for each tumor sample. The procedure followed was the same as that outlined for the foregoing test. The results shown in Table 2 indicate that the magnitude of fall in viscosity induced by this agent is the same for concentrations ranging from 1γ to 100γ. It may, therefore, be assumed that with above-threshold concentrations this polysaccharide exerts a maximal reduction in the viscosity of tumor cells. Furthermore, the mean viscosity values resulting from the two polysaccharides are practically the same, and the MVV due to colchicine does not vary greatly from these (Table 1). Hence it is

‡ International Equipment Co., Boston.

§ For definition, see Reference 14.

‖ Supplied by Dr. Hugh J. Creech, Lankenau Hospital Research Institute and the Institute for Cancer Research, Philadelphia.

¶ Supplied by Baxter Laboratories, Inc., Morton Grove, Ill.

# When 1.0γ amounts were needed for a test, the stock solutions were first diluted 1:10 (10γ/ml.), and 0.1 ml. of this concentration was used.

TABLE 1.—*Effects of Various Agents upon Protoplasmic Viscosity of Ehrlich Ascites Tumor Cells in Vitro*

| Agent* | No. Animals | Amount Used per Test | Mean Viscosity Valve (MVV) | Standard Error | t† | P |
|---|---|---|---|---|---|---|
| Untreated control | 30 | | 0.96 | 0.01 | | |
| 0.85% NaCl | 30 | 1.0 ml. | 0.96 | 0.02 | 0 | >0.99 |
| Piromen | 7 | 1.0 γ | 0.72 | 0.06 | 5.64 | <0.01 |
| MP5LL4 polysaccharide | 16 | 1.0 γ | 0.73 | 0.05 | 4.61 | <0.01 |
| Colchicine | 17 | 0.04 γ | 0.62 | 0.04 | 4.92 | <0.01 |

*Duration of exposure: 15 to 30 minutes before centrifugation.
† *t* value for 0.01 probability level.

probable that all three substances cause a maximal fall in the protoplasmic viscosity under the conditions in which the test was performed.

(*b*) In Vivo Tests: For the determination of changes in viscosity values resulting from intraperitoneal injections of the two polysaccharides and colchicine, male Strong A mice with a tumor growth of 7 to 14 days were used. After the samples for base counts of polar cells were drawn from the abdominal cavity, the animals were given various concentrations of the test substances intraperitoneally as outlined below:

MP5LL4 polysaccharide: Total doses of 10γ to 50γ (of 100γ/ml.*) per mouse

Piromen: Total doses of 10γ to 50γ (of 100γ/ml.*) per mouse.

Colchicine: 1γ per gram body weight (of 80γ/ml.* or $2.01\times10^{-4}$M).

Fifteen minutes after the test dose, each animal was again aspirated to determine the viscosity values of the tumor cells. The results are shown in Table 3. The MVV for all three agents shows a significant fall. Again it is noted that the magnitude of fall is practically the same for all substances used. The variations in the dose of the two polysaccharides used for this study yielded no significant difference in the MVV at 15 minutes.

Since the viscosity-reducing activity of the polysaccharides and colchicine was demonstrated within a short period following their intraperitoneal injections, it was desirable next to establish the duration of activity exhibited by these substances upon the cells. At least two factors are believed to influence the length of time in which the test agents remain active on the tumor cells in vivo. The first is the rate of elimination of the test material either by excretion and/or by detoxification by the host. The second is the toxicity of the substances and their lethal effects on the host, as well as on the tumor cells.

Although no attempt was made to investigate the rate of elimination of the test substances by the host, several experiments were designed to obtain sequential samples of the tumor following single intraperitoneal injections of the agents. Through such experiments we were able to establish that Piromen at moderately toxic concentrations

* Dissolved in 0.85% NaCl solution.

TABLE 2.—*Effects of Variable Concentrations of MP5LL4 Polysaccharide on Viscosity Values of Tumor Cells Tested in Vitro*

| Animal No. | Viscosity Values (VV) | | | |
|---|---|---|---|---|
| | Saline Control | Concentration of MP5LL4 Polysaccharide | | |
| | | 1.0 γ | 10.0 γ | 50.0 γ |
| 1 | 0.85 | 0.80 | 0.69 | 0.68 |
| 2 | 1.05 | 0.78 | 0.69 | 0.69 |
| 3 | 0.99 | 0.83 | 0.77 | 0.85 |
| 4 | 0.98 | 0.81 | 0.81 | 0.83 |
| 5 | 1.05 | 0.89 | 0.91 | 0.88 |
| 6 | 0.89 | 0.87 | 0.66 | 0.68 |
| 7 | 0.98 | 0.78 | 0.78 | 0.77 |
| MVV | 0.96 | 0.78 | 0.76 | 0.76 |

TABLE 3.—*Effects of Single Injection of Various Agents upon the Protoplasmic Viscosity of Tumor Cells in Vivo*

| Agent† | Duration after I.P. Injection | | | | | |
|---|---|---|---|---|---|---|
| | 0 Min. (Pretreatment)* | | | 15 Min. | | |
| | No. Ans. | MVV | Standard Error | No. Ans. | MVV | Standard Error |
| Saline control | 12 | 0.98 | 0.01 | 12 | 0.97 | 0.01 |
| Piromen | 5 | 0.94 | 0.03 | 5 | 0.70 | 0.08 |
| MP5LL4 polysaccharide | 18 | 0.97 | 0.02 | 18 | 0.66 | 0.04 |
| Colchicine | 11 | 0.99 | 0.01 | 9 | 0.66 | 0.04 |

* For initial or base polar-cell counts, aliquots were withdrawn immediately before injection of test agents.
† Dosage varied: consult text.

(50γ-100γ per milliliter) exerts a viscosity-reducing effect for three hours or less. Experiments using small single doses (5γ to 10γ) of MP5LL4 polysaccharide showed a similar effect. When higher single doses of this polysaccharide, in the range of 20γ to 50γ, were used, the duration of viscosity fall, on the whole, was prolonged to somewhat less than five hours. Doses of from 50γ to 100γ did not alter this result to any great extent but were found very toxic and lethal to many hosts bearing the tumor. One injection of colchicine in the amount previously specified was found effectively to maintain the viscosity-reducing effect on tumor cells for approximately 24 hours.

In view of the short duration of activity exhibited by Piromen, this substance was considered unsuitable and was eliminated from further in vivo investigations. Additional studies were made only on MP5LL4 polysaccharide and colchicine.

Mortality studies of S. marcescens polysaccharide by Creech and Hankwitz [18] were based on effects noted in Swiss mice. In our own work the lethal dose in Strong A mice with tumor was found to be much smaller than that reported for normal mice by these authors. Since a large survival rate of mice bearing the tumor was considered essential in our project, single doses above 50γ to 75γ were avoided, for they were usually fatal to the animals. For all subsequent studies of MP5LL4 polysaccharide, single doses of 50γ or less per mouse were selected.

*2. Standard Growth Curve of Ehrlich Ascites Tumor in Male Strong A Mice.*—Before we attempted to study the correlation of the viscosity values of tumor cells with the mitotic indexes following intraperitoneal injections of MP5LL4 polysaccharide or colchicine, it was necessary to establish a standard growth curve of Ehrlich ascites carcinoma in male Strong A mice. The computation of a theoretical multiplication rate of the tumor cells at any given instant during the active growth phase would then be possible.[15] The theoretical cell-multiplication curve obtained in this manner could be used to determine whether an elevation in the mitotic index (MI) represents a response to the stimulatory activity of the test substance, and, therefore, a true increase in the mitotic division, or whether such an apparent rise is due to the accumulation of cells arrested in some phase of mitosis. Should the former condition prevail, the MI would not necessarily correspond to the calculated multiplication curve, which theoretically represents the number of daughter cells arising from 100 cells for various time intervals (several hours) in a tumor of known age. On the other hand, if the rise in MI is due to arrested mitoses, the values should fall on the theoretical curve.

In addition to determining the theoretical cell-multiplication curve, the standard growth curve is of considerable value for comparing the effects of MP5LL4 polysaccharide or colchicine on the over-all

growth rate of the Ehrlich ascites carcinoma.

With the procedures described by Klein and Révész,[15] Révész and Klein,[19] and Patt, Blackford, and Drallmeier,[20] the necessary data for the standard growth curve were obtained from 34 Strong A mice 3 to 6 months old. Initial inocula of 2,000,000 viable Ehrlich ascites carcinoma cells were given intraperitoneally, and the growth data were obtained by killing two or three mice at intervals, as shown in Figure 1. In our own work, corrections were made for nonviable cells and the presence of leukocytes in the ascitic-fluid suspensions for each sample. These corrections were considered important, since MP5LL4 polysaccharide and colchicine are toxic and experiments were designed for their intraperitoneal use on mice with tumor, as will be discussed in another communication.

The standard growth curve obtained for Strong A mice is shown in Figure 1. Each point represents a mean of values obtained from two or three mice. The slope of this curve is nearly identical with the one obtained by Klein and Révész,[15] in which 1,800,000-cell inocula of Ehrlich ascites tumor were given. These results indicate that the growth pattern of the tumor in Strong A mice is essentially the same as that obtained in mice used by Klein and Révész. It can then be assumed that their regression data for the straight-line relationship between the cube roots of the tumor cell numbers and the time after inoculation would be the same for our own tumor. Using the mean regression coefficient value of 3.87, described by Klein and Révész, for the inoculum of 1,800,000 cells and their formula for velocity coefficient,† [15] we were able to calculate the theoretical rate of multiplication arising from 100 cells per unit of time. For a tumor growth of nine days, the number of daughter cells arising from 100 parent cells per hour can be expressed as the steep diagonal line shown in Figure 2, disregarding for the moment the flat portion of the

† $C=\frac{3b}{b(t\text{-}to)+\sqrt[3]{No}}$

where $C$=the velocity coefficient (multiplied by 100=percentage multiplication rate); $b$, mean regression coefficient (3.87 for initial tumor inoculum of $1.8\times10^6$); $t\text{-}to$, time that elapsed since the growth has started; $No$, tumor-cell number at start of the growth.

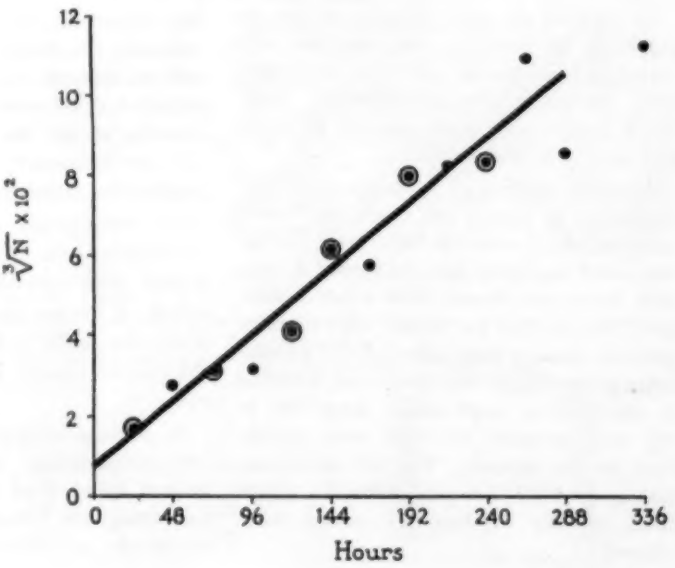



Fig. 1.—Growth curve of Ehrlich ascites tumor during the cube-root phase after an initial inoculum of 2,000,000 tumor cells. $N$ represents the total viable tumor cells per animal; the solid circle represents a mean value derived from two mice, and a circle within a circle represents a mean value derived from three mice.

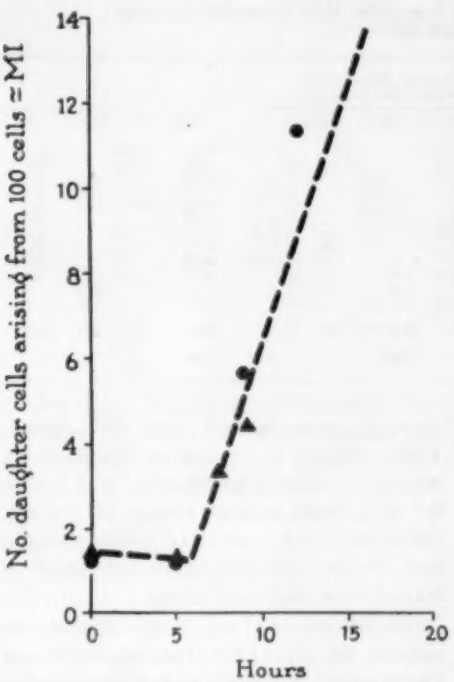



Fig. 2.—Comparison of mitotic indexes of MP5LL4-polysaccharide-treated tumor cells (triangles) and colchicine-treated tumor cells (circles) with the theoretical multiplication rate of untreated cells (broken line).

curve represented in the first six hours. The reason for including the "inactive period" of growth during the first six hours will become apparent when the problem of the effects of MP5LL4 polysaccharide and colchicine on the MI is taken up later.

3. *Correlation of Structural Viscosity with Mitotic Index During Cube Root Phase of Tumor Growth.*—The technique for the determination of MI consisted of making smears of the ascitic-fluid samples on clean microscopic slides, as in the preparation of blood smears. Heavy smears were found more suitable than thin ones. The slides were dried in air and stained by a modification of the acetic acid-orcein method. A fresh preparation of staining Solution B (described by Tjio and Levan [21]) in a Coplin jar was heated in a water bath maintained at 60-70 C. After being dried thoroughly, the slides smeared with the ascitic-fluid suspension were stained in this solution for 5-10 seconds and carefully rinsed in running tap water. The slides were dried and 1000 tumor cells were counted randomly under the dark-contrast oil-immersion lens of a phase-contrast microscope. In this examination, various stages of the mitotic figure ‡ were identified and their percentages were determined. The MI was expressed as the percentage of cells undergoing mitosis in a total count of 1000 cells.

Klein and Révész [15] showed that the mean MI of Ehrlich ascites carcinoma (initial inoculum of 1,800,000 cells) decreased progressively from 4.9, at 45 hours of growth, to 1.3, at 330 hours. In the same study the MI of 6½ to 14 days' growth ranged from 1.3 to 2.5. Since, in our own studies, the ages of the tumor ranged from 7 to 14 days for a total of 30 mice (Section (*a*) below), it was reasonable to speculate that the upper limit of the MI would probably not exceed 2.5. This belief was substantiated by our mitotic counts, in which the MI averaged 1.2 and ranged from 0.4 to 2.5 (Tables 5, 7, and 9, under Column 0).

(*a*) Alterations in Protoplasmic Viscosity and Mitotic Index of Tumor Cells Following Single Intraperitoneal Injection of Colchicine: For this experiment, male Strong A mice given an intraperitoneal inoculum of 0.2 ml. of the ascites-tumor suspension § were permitted to develop their tumors for 7 to 14 days. The animals were selected randomly after the abdominal distention was considered sufficient for several samples of 0.5 ml. each. Although 14-day-old tumors actually represent the very end of the cube-root growth phase or the beginning of the terminal phase (asymptote), four animals with tumor of this age were used in this study because the rate of growth seemed slightly delayed in these mice.

‡ Identification was limited only to late prophase, metaphase, and anaphase-telophase.

§ Cell counts of the inoculum were not done.

TABLE 4.—*Viscosity Values of Tumor Cells from Mice Given One Injection of Colchicine*

| Animal No. | Animal Wt., Gm. | Viscosity Values (VV) (Hours After Injection) 0 | ¼ | 2½ | 5 | 7 | 9 | 12 | 17 | 20 | 24 | 27½ | 36 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 28.6 | 1.00 | 0.65 | 0.67 | 0.65 | -- | -- | -- | -- | -- | 0.96 | -- | -- |
| 2 | 63.9 | 0.98 | 0.52 | 0.58 | 0.54 | -- | -- | -- | -- | -- | -- | -- | -- |
| 4 | 54.5 | 1.01 | 0.75 | -- | -- | -- | -- | -- | 0.74 | 1.03 | -- | -- | -- |
| 5 | 48.5 | 0.99 | 0.68 | -- | -- | -- | -- | -- | 0.91 | -- | -- | -- | -- |
| 6 | 45.0 | -- | 0.69 | -- | -- | -- | -- | -- | 0.65 | 0.63 | 0.85 | -- | -- |
| 7 | 53.0 | 1.02 | 0.81 | -- | -- | -- | -- | -- | 0.71 | 1.02 | -- | -- | -- |
| 10 | 54.0 | 1.08 | -- | -- | -- | -- | -- | -- | 1.02 | -- | -- | -- | -- |
| 11 | 42.6 | 0.96 | -- | -- | -- | -- | -- | 0.38 | -- | -- | -- | -- | -- |
| 12 | 49.1 | 0.96 | -- | -- | -- | -- | -- | 0.73 | 0.59 | 0.60 | -- | -- | 1.06 |
| 13 | 58.3 | 0.98 | 0.62 | -- | 0.61 | -- | 0.64 | -- | -- | -- | -- | -- | -- |
| 14 | 57.6 | 0.96 | 0.74 | 0.83 | 0.56 | 0.57 | 0.62 | -- | -- | -- | -- | 0.92 | -- |
| 15 | 50.9 | 0.93 | 0.52 | 0.60 | 0.56 | 0.57 | 0.55 | -- | -- | -- | -- | 1.01 | -- |
| Mean | | 0.99 | 0.66 | 0.67 | 0.58 | 0.57 | 0.60 | 0.62 | 0.77 | 0.82 | 0.90 | 0.96 | 1.06 |
| Standard error | | 0.01 | 0.04 | 0.05 | 0.01 | -- | 0.04 | -- | 0.07 | 0.14 | -- | -- | -- |

For the performance of the test, the control sample was drawn from each mouse; smears were made and stained with HCl-acetic acid-orcein mixture, and the viscosity values were determined as previously described. The animals were then weighed and given intraperitoneal injection with 1.0γ of colchicine per gram of body weight from 80γ/ml. of stock solution. Samples were drawn at various intervals from 15 minutes to 36 hours after the injection of colchicine, as shown in Tables 4 and 5. The values for VV and MI were determined for each sample taken at these intervals (Tables 4 and 5).

The results in Table 4 show that the protoplasmic viscosity (MVV) of tumor cells fall rapidly from a value of 0.99 to 0.66. It remains low for about 12 hours; then a gradual rise occurs until a normal value is again reached after 27½ hours. Table 5 shows that the mean mitotic index remains essentially unchanged for 5 hours, but in 7 hours a rapid rise in MI occurs, and it reaches a peak at 12 hours. After a slow decline, the MI again returns to a normal level within 30 hours.

(*b*) Effects of Two Intraperitoneal Injections of MP5LL4 Polysaccharide on Protoplasmic Viscosity and Mitotic Index of Tumor Cells: The preceding in vivo tests indicate that a single, sublethal intraperitoneal dose of MP5LL4 polysaccharide results in a rapid fall of tumor-cell viscosity. It was shown that this state of diminished viscosity usually has a duration of somewhat less than five hours. Since the sequential in vivo study of the tumor mitotic index in animals treated with colchicine showed a period of inactivity or latency for the first

TABLE 5.—*Mitotic Indexes of Tumor Cells from Mice Given One Injection of Colchicine*

| Animal No. | Animal Wt., Gm. | Mitotic Index (MI) (Hours) 0 | 2½ | 5 | 7 | 9 | 12 | 17 | 20 | 24 | 30-41 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 28.6 | 1.1 | 1.4 | 1.3 | -- | -- | -- | -- | -- | -- | -- |
| 2 | 63.6 | 0.5 | 0.3 | 0.5 | -- | -- | -- | -- | -- | -- | -- |
| 4 | 54.5 | 1.2 | -- | -- | -- | -- | -- | 14.5 | 9.1 | 6.1 | -- |
| 5 | 48.6 | 1.3 | -- | -- | -- | -- | -- | 5.5 | -- | -- | -- |
| 6 | 45.0 | 0.8 | -- | -- | -- | -- | -- | 9.9 | 10.2 | 5.1 | -- |
| 7 | 53.0 | 0.4 | -- | -- | -- | -- | -- | 10.3 | 3.7 | -- | 0.9 |
| 10 | 54.0 | 1.1 | -- | -- | -- | -- | -- | 4.8 | -- | -- | -- |
| 11 | 42.6 | 1.4 | -- | -- | -- | -- | 15.6 | 1.3 | -- | -- | 0.5 |
| 12 | 49.1 | 0.7 | -- | -- | -- | -- | 5.4 | 12.7 | 10.0 | -- | 1.0 |
| 13 | 58.3 | 0.9 | -- | 0.6 | -- | 0.8 | -- | -- | -- | -- | -- |
| 14 | 57.6 | 1.3 | 1.0 | 1.3 | 2.2 | 7.9 | -- | -- | -- | -- | 1.9 |
| 15 | 50.9 | 2.1 | -- | 1.6 | 1.7 | 8.3 | 13.2 | -- | -- | -- | 2.3 |
| Mean | | 1.2 | 0.9 | 1.1 | 2.0 | 5.7 | 11.4 | 8.6 | 8.2 | 5.6 | 1.3 |
| Standard error | | 0.1 | 0.4 | 0.2 | -- | 2.9 | 3.5 | 1.9 | 1.8 | -- | 0.4 |

TABLE 6.—*Viscosity Values of Tumor Cells from Mice Given Two Injections of MP5LL4 Polysaccharide*

| Animal No. | Total Dose MP5LL4, γ | Viscosity Values (VV) (Hours After First Injection) 0 | ¼ | 2½ | 5 | 5¼ | 7½ | 9 | 12 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 60 | 0.94 | 0.47 | -- | 0.89 | -- | -- | 0.60 | -- | -- |
| 2 | 60 | 0.99 | 0.57 | -- | 1.00 | -- | -- | -- | -- | -- |
| 3 | 60 | 0.98 | 0.79 | 0.83 | 1.01 | 0.84 | 0.96 | 1.00 | -- | 1.00 |
| 4 | 60 | 0.97 | 0.65 | 0.64 | 0.97 | 0.89 | 0.87 | 0.89 | -- | 1.00 |
| 25 | 60 | 0.95 | 0.82 | -- | 1.08 | 0.79 | 0.95 | 1.03 | 0.93 | 0.97 |
| 17 | 40 | 1.03 | 0.76 | 0.67 | 0.97 | 0.72 | -- | 0.60 | 1.02 | 0.97 |
| 19 | 40 | 1.02 | 0.67 | 0.63 | 0.96 | 0.66 | -- | 1.00 | 0.98 | 1.00 |
| 20 | 40 | 0.96 | 0.68 | -- | 0.96 | 1.04 | 0.88 | 0.95 | -- | 0.94 |
| 21 | 40 | 0.93 | 0.62 | -- | 1.01 | 1.04 | 0.78 | 0.98 | -- | 0.97 |
| 23 | 40 | 1.00 | 0.72 | -- | 0.90 | 0.64 | 0.93 | 1.04 | 1.02 | 1.03 |
| Mean | | 0.98 | 0.65 | 0.69 | 0.99 | 0.83 | 0.90 | 0.90 | 0.99 | 0.99 |
| Standard error | | 0.01 | 0.03 | 0.05 | 0.02 | 0.06 | 0.03 | 0.06 | 0.03 | 0.01 |

six hours,‖ it was considered possible that a similar lag might be present during the polysaccharide treatment. It was therefore decided that an administration of a second injection of the polysaccharide would be indicated five hours after the injection of the initial dose in order to maintain a state of reduced protoplasmic viscosity beyond the period of latency. The procedure followed for this study was the same as outlined for colchicine, except that two doses of the test agent were given with an interval of five hours.

In considering the validity of results obtained from the tests outlined above, it is imperative to keep in mind the toxic effects upon the hosts of such an agent as MP5LL4 polysaccharide. For the study of the phenomenon of arrested cell division, the first requirement is that the mitotic activity continues unhindered up to the stage of blockage. To be assured that the tumor cells participate in the process of active growth, and thus in active mitotic division, it is essential that the host be maintained in a reasonably good state of health to provide the cells with an adequate physiologic milieu for such basic needs as the provision of nutriments, gaseous exchange, etc. In consequence, we established as a requirement that the host must survive the toxic effects of the polysaccharide for at least 24 hours after the first injection. The animals that died prior to this period were discarded from the study. A total dose of 60γ (two injections of 30γ) had to be reduced to 40γ (in two equal doses) in order to reduce the mortality in this series (Tables 6 and 7).

The results are presented in Tables 6 and 7. The MVV after the first injection is

‖ Latent period of six hours was selected, since the MI at five hours showed no rise but a slight elevation occurred at seven hours. This is in agreement with Levan.[17]

TABLE 7.—*Mitotic Indexes of Tumor Cells in Mice Given Two Injections of MP5LL4 Polysaccharide*

| Animal No. | Total Dose MP5LL4, γ | Mitotic Index (MI) (Hours After First Injection) 0 | 5 | 7½ | 9 | 12 | 24 |
|---|---|---|---|---|---|---|---|
| 1 | 60 | 1.4 | 0.7 | -- | 5.6 | -- | -- |
| 2 | 60 | 0.8 | 1.0 | -- | 5.2 | -- | -- |
| 3 | 60 | 1.5 | 0.9 | 2.4 | 4.5 | 2.0 | 0.8 |
| 4 | 60 | 1.4 | 0.8 | 3.4 | 5.1 | 1.6 | 1.2 |
| 25 | 60 | 2.5 | 0.7 | 2.7 | 4.2 | 3.1 | 1.5 |
| 17 | 40 | 1.8 | 1.0 | -- | 4.6 | 2.9 | 1.7 |
| 19 | 40 | 1.0 | 1.4 | -- | 2.3 | 1.4 | 0.6 |
| 20 | 40 | 0.6 | 3.1 | 6.1 | 4.9 | 1.8 | 1.2 |
| 21 | 40 | 1.0 | 1.1 | 2.8 | 5.1 | 2.8 | 0.9 |
| 23 | 40 | 1.6 | 1.1 | 1.6 | 2.6 | 0.9 | 1.7 |
| Mean | | 1.4 | 1.2 | 3.3 | 4.4 | 2.1 | 1.2 |
| Standard error | | 0.2 | 0.2 | 0.7 | 0.1 | 0.3 | 0.2 |

TABLE 8.—*Viscosity Values of Tumor Cells from Mice Given One or Two Injections of 0.85% Saline Intraperitoneally*

| Animal No. | Total Saline Injected, Ml. | Viscosity Values (VV) (Hours After First Injection) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | ¼ | 2½ | 5 | 5¼ | 7½ | 9 | 17 | 20 | 24 |
| 5* | 0.3 | 0.99 | 0.99 | 0.96 | 0.99 | -- | -- | 0.99 | -- | -- | -- |
| 8C* | 1.63 | 0.96 | 0.95 | -- | -- | -- | -- | -- | 0.97 | 0.97 | 0.97 |
| 26 | 0.6 | 0.98 | 1.02 | -- | 1.05 | 0.98 | 0.98 | -- | -- | -- | 0.95 |
| 27 | 0.6 | 1.03 | 1.00 | -- | 0.96 | 0.99 | 1.04 | -- | -- | -- | 0.99 |
| 28 | 0.6 | 1.04 | 0.99 | -- | 1.00 | 1.01 | 1.05 | -- | -- | -- | 1.00 |
| 29 | 0.6 | 0.98 | 0.96 | -- | 1.01 | 1.01 | 1.00 | -- | -- | -- | 1.04 |
| 30 | 0.6 | 0.98 | 1.01 | -- | 0.98 | 0.99 | 0.98 | -- | -- | -- | 0.99 |
| 31 | 0.6 | 1.03 | 1.00 | -- | -- | -- | -- | -- | -- | -- | -- |
| Mean | | 1.00 | 0.99 | 0.96 | 1.00 | 0.99 | 1.01 | 0.99 | 0.97 | 0.97 | 0.99 |
| Standard error | | 0.01 | 0.01 | -- | 0.02 | 0.02 | 0.02 | -- | -- | -- | 0.02 |

*One injection only; others received two injections.

seen to fall almost immediately; however, the effect is not prolonged, and the tumor cells regain their normal state of rigidity within five hours. The second injection, again, causes an immediate fall in MVV, but the magnitude of fall is less than that observed after the first dose. This state of slightly diminished viscosity lasts about nine hours. Concurrent samples of mitotic counts show that the mean MI rises from a relatively constant base value of 1.4, for the first five hours, to a mean value of 4.4 in nine hours. This peak is followed by a recession until a normal mean value is again reached by 24 hours. In two mice, Nos. 19 and 23, however, the increase in the MI is very slight and of questionable significance at nine hours.

(*c*) Effects of One or Two Intraperitoneal Injections of 0.85% NaCl on Protoplasmic Viscosity and the Mitotic Index of Tumor Cells: To check against the possible effects of the diluent, 0.85% NaCl, on the viscosity and the MI of tumor cells, tests were repeated as outlined in the two preceding sections. Two mice with tumor were given one injection of isotonic saline solution, and the remainder, two injections at an interval of five hours. The volumes of the solution were varied to correspond with the range of the minimum and maximum volumes used for colchicine and MP5LI.4 polysaccharide.

The results in Tables 8 and 9 show that no significant change in MVV or MI occurs when 0.85% NaCl solution alone is used.

4. *C-Mitosis.*—The ascitic-fluid smears stained for mitotic index counts were used to determine the percentage of mitotic figures resembling C-mitosis. In his studies with colchicine on the mitosis of Ehrlich ascites carcinoma, Levan [17] described two effects on mitosis, the initial C-mitosis and the scattered C-mitosis. The initial type is described as resulting from the effect of colchicine on the dividing cells, in the phases

TABLE 9.—*Mitotic Indexes of Tumor Cells from Mice Given One or Two Injections of 0.85% Saline Intraperitoneally*

| Animal No. | Total Saline Injected, Ml. | Mitotic Index (MI) (Hours After First Injection) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2½ | 5 | 7½ | 9 | 12 | 17 | 24 |
| 5* | 0.3 | 0.6 | 0.4 | 0.9 | -- | 2.5 | 1.3 | -- | -- |
| 8C* | 1.63 | 1.0 | -- | -- | -- | -- | -- | 1.2 | 1.3 |
| 26 | 0.6 | 1.3 | -- | 0.7 | 1.1 | 1.1 | 1.2 | -- | 1.2 |
| 27 | 0.6 | 1.0 | -- | 0.5 | 0.6 | 0.6 | 1.5 | -- | 1.3 |
| 28 | 0.6 | 1.4 | -- | 1.1 | 1.0 | 1.9 | 1.8 | -- | 1.8 |
| 29 | 0.6 | 0.8 | -- | 0.8 | 0.7 | 0.5 | 0.5 | -- | 0.4 |
| 30 | 0.6 | 0.4 | -- | 0.5 | 0.4 | 0.4 | 0.5 | -- | 0.3 |
| 31 | 0.6 | 0.9 | -- | -- | -- | -- | -- | -- | 1.1 |
| Mean | | 0.9 | 0.4 | 0.8 | 0.8 | 1.2 | 1.1 | 1.2 | 1.1 |
| Standard error | | 0.1 | -- | 0.1 | 0.1 | 0.4 | 0.2 | -- | 0.2 |

* One injection only; others received two injections.

TABLE 10.—*Percentages of C-Mitosis Resulting from MP5LL4 Polysaccharide and Colchicine Treatments in Vivo*

| Test Agent | Type of Mitosis,* % | Hours After First Injection | | | | | Total Mitotic Cells Counted† |
|---|---|---|---|---|---|---|---|
| | | 0 | ¼ | 5 | 7½ | 24 | |
| MP5LL4 polysaccharide | Normal | 91 | 60 | 50 | 12 | 48 | 1102 |
| | C-mitosis | 9 | 40 | 50 | 88 | 52 | |
| Colchicine | Normal | 95 | 43 | -- | -- | 12 | 669 |
| | C-mitosis | 5 | 57 | -- | -- | 88 | |

* Prophases are not included in the computation, as it is too difficult to distinguish between the normal and the C varieties.
† Ranged from 163 to 332 cells for each time interval.

associated with spindle activity, at the time colchicine is introduced. The scattered type develops after a longer exposure to the drug, and, characteristically, the chromosome pairs are seen to be distributed widely throughout the cell. In our present study no attempt was made to separate the clustered from the scattered types. Both groups were merely identified and counted under the category of C-mitosis. The percentages of C-mitosis resulting from MP5LL4 polysaccharide and from colchicine for periods representing a lapse of time of from 15 minutes to 24 hours are tabulated in Table 10. Our data represent only a small population of cells in division, but they do show a striking increase in the percentage of C-mitoses within 15 minutes following the administration of the polysaccharide or colchicine. A similar effect was noted by Levan as early as 10 minutes after the injection of colchicine. The highest level of C-mitosis attained with MP5LL4 polysaccharide is 88%, occurring seven and one-half hours after the initial dose. By 24 hours, even with the administration of

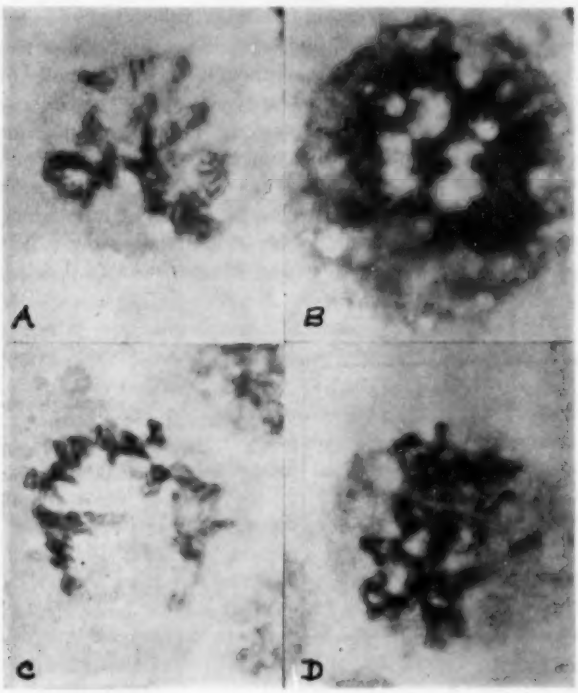



Fig. 3.—*A* and *B* are examples of initial or condensed forms of C-Mitosis; *C* and *D* represent scattered varieties. *A* and *C* were treated with MP5LL4 polysaccharide; *B* and *D* were treated with colchicine. Phase contrast.

the second injection of polysaccharide, the C-mitosis level falls to 52%.

Using a dose of 1.5γ of colchicine per gram of body weight, Levan obtained 57.2% C-mitosis at 30 minutes. In our own series, with 1.0γ per gram of body weight, a comparable level of 57% is noted in 15 minutes. In mice treated with colchicine the peak of C-mitosis is apparently reached in about an hour [17] and remains elevated for 24 hours or more, depending upon the dose. From these results, it is apparent that colchicine is more effective than MP5LL4 polysaccharide in prolonging the induction of C-mitosis. Examples of C-mitosis due to the polysaccharide and colchicine are shown in Figure 3.

## Comment

The slope of the curves obtained by plotting the rise in the mean mitotic indexes of the tumor against time, after the administration of MP5LL4 polysaccharide and colchicine, is seen to be nearly the same as the slope for the computed theoretical curve. If a level line representing a six-hour lag period is added in front of the slanting theoretical curve, so that it would correspond to the period of relative inactivity or the latent period of the mitotic index exhibited by the tumor cells exposed to the test agents, then the correlation between the values of the mitotic index and the theoretical curve is found to be excellent (Fig. 2). Should this rise represent a true increase in the mitotic activity by stimulation, then it would be expected that the tumor growth will accelerate, but such results were not seen. We believe that MP5LL4 polysaccharide, as well as colchicine, is responsible for the apparent increase of the mitotic index of the Ehrlich ascites carcinoma cells, but that this is brought about by the arrest of cell division. In the case of cells treated with the polysaccharide, the number of metaphases per 1000 cells increased from a mean of 8.9 for the controls to 29.9 during the peaks of elevation in the mitotic index. A similar, but greater, increase occurred in cells treated with colchicine, and the mean number of metaphases rose to 95.7 per 1000 cells. From these data it can be concluded that MP5LL4 polysaccharide, like colchicine, arrests the dividing tumor cells at metaphase.

Mean values of the mitotic index and the relative viscosity are reproduced on a composite graph for the tumor treated with MP5LL4 polysaccharide in Figure 4. Owing to a transient effect of the polysaccharide on the MVV following the initial dose, a second injection was repeated five hours later. Although the magnitude of fall in the viscosity after the second dose is slight, the state of reduced protoplasmic rigidity is prolonged to about the ninth hour. After five to six hours, the mean mitotic index rises to a peak at nine hours. Lippman,[22] in her work relating the effects of the polysaccharide heparin on the Ehrlich

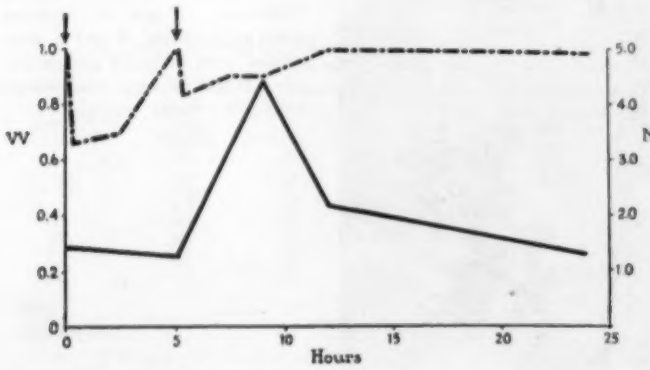



Fig. 4.—Graphic representation of mean viscosity values (VV) and mean mitotic indexes (MI) of tumor cells in MP5LL4-polysaccharide-treated mice. Arrows indicate the time of polysaccharide injections.

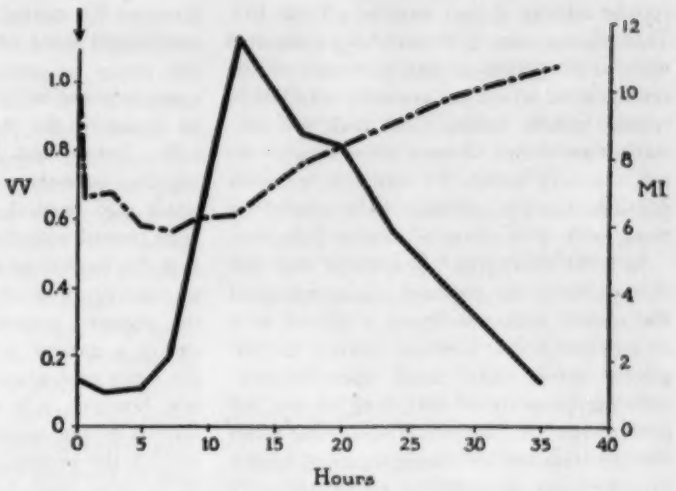



Fig. 5.—Graphic representation of mean viscosity values (VV) and mean mitotic indexes (MI) of tumor cells in colchicine-treated mice. Arrow indicates the time of colchicine injection.

ascites tumor, found a statistically significant reduction in the mitotic index of tumor cells sampled mostly from one to three hours after the injection of this test material. Since the action of heparin as a mitosis inhibitor is believed to depend on its activity in lowering the viscosity of the cell protoplasm,[1, 5] it is difficult to see why the mitotic index should be suppressed when the action of such agents is presumed to be in the prevention of mitotic gelation, and hence upon the formation of spindle fibers. It is possible that an increase in the mitotic index (metaphase arrest) would be demonstrable if the tumor samples were checked after a longer lapse in time following the injection of heparin. Perhaps a latent period could also be assumed for cells treated with heparin, and, consequently, samples of cells taken during the first few hours reveal little change in the mitotic index.

The administration of colchicine results in a rather prolonged reduction of MVV, and the effect on the MI is more pronounced (Fig. 5). As in the case of MP5LL4 polysaccharide treatment, a latent period of about six hours is again noted. Elevation of the MI appears to be in some way related to the fall in MVV, but the reason for the occurrence of the latent period is not known. Levan [17] believes that the lag period of six hours, noted during his studies of the mitotic index with colchicine treatment of Ehrlich ascites carcinoma, was due to the "shock" of cells being initially exposed to the drug. It is quite conceivable that a readjustment period is required by these cells before they are able to resume their mitotic activities after an introduction of extraneous and toxic substances.

That the solvent (0.85% NaCl) used with the polysaccharide and colchicine did not influence the MVV or MI is clearly shown in Tables 8 and 9. The chemically unrelated test substances investigated here are, therefore, responsible for at least two effects on Ehrlich ascites carcinoma cells: (1) the lowering of protoplasmic viscosity, and (2) the inhibition of cell division through metaphase arrest. These two effects, we believe, are causally related, and our results further substantiate the observations of others on certain nonmammalian eggs and plant cells that were treated with colchicine and with polysaccharides derived from different biological sources.[1-3,9-18,23]

Within 15 minutes following the intraperitoneal administration of either MP5LL4 polysaccharide or colchicine the development of C-mitosis is observed in those tumor cells already undergoing division and exhibiting

spindle activity at that moment (Table 10). This phenomenon is theoretically associated with a disruption in the moorage of the centromeres, which are normally attached to certain spindle fibers. Since both test substances are shown to cause a lowering of the cell viscosity within 15 minutes, it seems possible that the spindles were solated by them, with a resultant C-mitosis induction.

In 1944 Östergren[9] postulated that colchicine alters the physical configuration of the spindle molecules from a fibrous to a corpuscular form; however, several investigators before him noted the viscosity-reducing property of this drug on the cell protoplasm.[10-12] Northen[13] favors the belief that dissociation or disaggregation, rather than a change in molecular configuration of the protein, is the primary cause of decrease in the protoplasmic viscosity resulting in cells treated with this drug. Mazia[24, 25] contends that the development of the mitotic apparatus represents a dual process: that of the formation of a gel and the condensation and orientation of certain parts of the gel corresponding to the visible fibers. Utilizing a technique for isolating the mitotic apparatus,[26] Mazia showed that sea urchin eggs treated with colchicine had condensed chromosomes that were seen to be embedded in a mass of disorganized gel rather than in association with visible spindle fibers.[24, 25] According to this concept, the mechanism responsible for the orderly condensation and orientation of protein molecules is primarily attacked by the action of colchicine, for a gel formation was, at least, apparent. The gel in this instance was recognized only after "selective solubilization" by a dispersing agent, and whether it actually existed prior to such a treatment is not absolutely certain. Since the determination of viscosity by displacement of lipid granules under centrifugation in the cells of Ehrlich ascites tumor actually represents a measurement of the stiffness of the cell cortex,[14] it might be argued that local differences of viscosity in the inner cell mass would not be detected by such a technique. Northen[13] utilized the displacement by centrifugal force of mitotic figures of resting nuclei in meristematic cells of onion roots exposed to colchicine as an indicator of change in the structural viscosity of the cells. Beams and Evans[10] used a centrifugation procedure which displaced the interior and cortical granules in sea urchin eggs treated with colchicine. They observed that the movement of the interior granules of the eggs was affected more readily than the pigment granules in the cortex, indicating a greater reduction in viscosity of the inner protoplasm. None of these methods, however, tells us anything of gelation limited to the mitotic apparatus itself, the rest of the protoplasm remaining liquefied. It is true that local differences in the physical change that occurs in the inner protoplasm will not be detected by these centrifugation methods. Nevertheless, it would appear that the main effect of colchicine, as well as of S. marcescens polysaccharide, is to reduce the protoplasmic viscosity of the entire cell. It is possible that the cortex of the cells is more refractory to the viscosity changes by these substances than the interior protoplasm, as shown by Beams and Evans with colchicine. Should this be the case for Ehrlich ascites tumor cells, the measurable difference in the lowering of viscosity in the cell interior might be even greater than the values we have presented for the changes observed in the cortex. The eventual outcome of the altered physical state induced by these test substances is apparently mirrored in the interference of spindle formation during cell division, because mitotic gelation under these conditions will not ordinarily develop.

## Summary

Both colchicine and Serratia marcescens polysaccharide were shown to reduce the protoplasmic viscosity of Ehrlich ascites tumor cells. A single intraperitoneal injection of colchicine into mice bearing the tumor induced a fall in the tumor-cell viscosity which lasted for approximately 24

hours, while one injection of the bacterial polysaccharide was effective for a little less than 5 hours. With either agent, no change of the mitotic index (MI) was noted for six hours (latent period). In the case of the polysaccharide, a second injection was needed to prolong the lowered viscosity in cells beyond the duration of the latent period. Under these conditions, the mitotic index rose from a mean of 1.2 to maximal peaks of 11.4 in 12 hours for colchicine and 4.4 in 9 hours for S. marcescens polysaccharide. With a fall in the protoplasmic viscosity, a gradual but distinct increase in the number of arrested mitoses was noted, whereas the opposite effect occurred when the viscosity values again returned to normal. These results lend support to the hypothesis that the development of spindle fibers is dependent upon the ability of the cell to maintain certain optimal states in the protoplasmic viscosity during mitosis.

Department of Pathology, Northwestern University Medical School, 303 E. Chicago Ave. (11).

## REFERENCES

1. Heilbrunn, L. V., and Wilson, W. L.: Effect of Heparin on Cell Division, Proc. Soc. Exper. Biol. & Med. 70:179-182, 1949.

2. Heilbrunn, L. V., and Wilson, W. L.: Effect of Bacterial Polysaccharide on Cell Division, Science 112:56-57, 1950.

3. Heilbrunn, L. V.; Wilson, W. L., and Harding, D.: The Action of Tissue Extracts on Cell Division, J. Nat. Cancer Inst. 11:1287-1298, 1951.

4. Heilbrunn, L. V.: An Outline of General Physiology, Ed. 3, Philadelphia, W. B. Saunders Company, 1952.

5. Heilbrunn, L. V.: The Dynamics of Living Protoplasm, New York, Academic Press Inc., 1956.

6. Heilbrunn, L. V.: An Experimental Study of Cell-Division: I. The Physical Conditions Which Determine the Appearance of the Spindle in Sea-Urchin Eggs, J. Exper. Zool. 30:211-237, 1920.

7. Heilbrunn, L. V.: Protoplasmic Viscosity Changes During Mitosis, J. Exper. Zool. 34: 417-447, 1921.

8. Heilbrunn, L. V., and Wilson, W. L.: Protoplasmic Viscosity Changes During Mitosis in the Egg of Chaetopterus, Biol. Bull. 95:57-68, 1948.

9. Östergren, G.: Colchicine Mitosis, Chromosome Contraction, Narcosis and Protein Chain Folding, Hereditas 30:429-467, 1944.

10. Beams, H. W., and Evans, T. C.: Some Effects of Colchicine upon the First Cleavage in Arbacia Punctulata, Biol. Bull. 79:188-198, 1940.

11. Yamaha, G., and Ueda, R.: Über die Wirkung des Kolchizins auf Spirogyra, Bot. & Zool. Syokubuta Oyobi Dobuta 8:1709-1714, 1940.

12. Wilbur, K. M.: Effects of Colchicine upon Viscosity of the Arbacia Egg, Proc. Soc. Exper. Biol. & Med. 45:696-700, 1940.

13. Northen, H. T.: Alterations in the Structural Viscosity of Protoplasm by Colchicine and Their Relationship to C-Mitosis and C-Tumor Formation, Am. J. Bot. 37:705-711, 1950.

14. Nishimura, E. T.; DiPaolo, J. A., and Hill, W. T.: Studies on the Viscosity of the Cytoplasm of Mouse Ehrlich Ascites Tumor Cells, A. M. A. Arch. Path. 59:487-494, 1955.

15. Klein, G., and Révész, L.: Quantitative Studies on the Multiplication of Neoplastic Cells *in Vivo*: I. Growth Curves of the Ehrlich and MCIM Ascites Tumor, J. Nat. Cancer Inst. 14: 229-277, 1953.

16. Lettré, H.; Krapp, R., and Ochsenschläger, M.: Wirkungen des Colchicins und N-Methylcolchicamids auf die Mitose der Zellen des Mäuse-Ascites-Tumors, Ztschr. Krebsforsch. 57:142-150, 1950.

17. Levan, A.: Colchicine-Induced C-Mitosis in Two Mouse Ascites Tumors, Hereditas 40:1-64, 1954.

18. Creech, H. J., and Hankwitz, R. F., Jr.: Biological Properties of Polysaccharide-Lipid Complexes Obtained from Serratia Marcescens and Escherichia coli, Cancer Res. 14:824-829, 1954.

19. Révész, L., and Klein, G.: Quantitative Studies on the Multiplication of Neoplastic Cells *in Vivo*: II. Growth Curves of Three Ascites Lymphomas, J. Nat. Cancer Inst. 15:253-273, 1954.

20. Patt, H. M.; Blackford, M. E., and Drallmeier, J. L.: Growth Characteristics of the Krebs Ascites Tumor, Proc. Soc. Exper. Biol. & Med. 83: 520-524, 1953.

21. Tjio, J. H., and Levan, A.: Some Experiences with Acetic Orcein in Animal Chromosomes, An. estac. exper. Aula Dei 3:225-228, 1954.

22. Lippman, M.: Effect of Heparin and Related Substances on Ascites Tumor in Mice, Master's Thesis, University of Pennsylvania, 1955.

23. Swann, M. M., and Mitchison, J. M.: Cleavage of Sea-Urchin Eggs in Colchicine, J. Exper. Biol. 30:506-514, 1953.

24. Mazia, D.: The Life History of the Cell, Am. Scientist 44:1-32, 1956.

25. Mazia, D.: Materials for the Biophysical and Biochemical Study of Cell Division, in Advances in Biological and Medical Physics, edited by J. H. Lawrence and C. A. Tobias, New York, Academic Press Inc., 1956, Vol. IV, pp. 69-118.

26. Mazia, D., and Dan, K.: The Isolation and Biochemical Characterization of the Mitotic Apparatus, Proc. Nat. Acad. Sc. 38:826-838, 1952.

# Hibernating Fat and Pheochromocytoma

M. M. MELICOW, M.D., New York

The presence of "hibernating fat" adjacent to a pheochromocytoma was first noted by us in April, 1953. Since then this type of fat has been observed in the pericapsular zone of 16 out of 18 cases operated on for pheochromocytoma at the Squier Urological Clinic of the Columbia-Presbyterian Medical Center. In some of the positive cases very little fat accompanied the specimens, and in a few the amount covered only one to several low-power fields, but even in the latter clusters of fat cells possessing "foamy" cytoplasm were present (Figs. 1, 2, 3). In one case no fat was included with the tumor, and in another the small amount of fat was of the adult variety only. A review of sections of 11 tumors removed during a preceding 13-year period, 1940 to April, 1953, showed hibernating fat to be present only in one case. In the remaining 10 cases no fat had been included in the tissue removed for sectioning. It is our opinion that the striking difference between the two consecutive groups of tumors is due to two factors: After our first observation, in April, 1953, of the presence of hibernating fat in close approximation to pheochromocytoma we requested that the surgeons include surrounding fat with the specimen. Prior to this they had been diligent in obtaining a clean dissection of the tumor from surrounding structures. In addition, we made certain to include in the blocks removed for study some of the pericapsular adipose tissue.

The hibernating fat seemed to be concentrated around the chromaffin tumors and was not seen in other places from which biopsies of fat were made. Thus in three patients whose pheochromocytomas were embedded in the "foamy" type of fat subcutaneous biopsies showed only adult fat. Biopsy specimens of the perirenal fat and that surrounding nearby lymph nodes were likewise "white" fat. A review of our cases of the adrenogenital and Cushing syndromes that were operated on for cortical hyperplasia or adenoma or carcinoma failed to show hibernating fat when fat had been included with the specimen. Examination of tissue surrounding a sympathicoblastoma of the adrenal medulla, and of that surrounding three renal neoplasms showed only adult fat (Figs. 4 to 9).

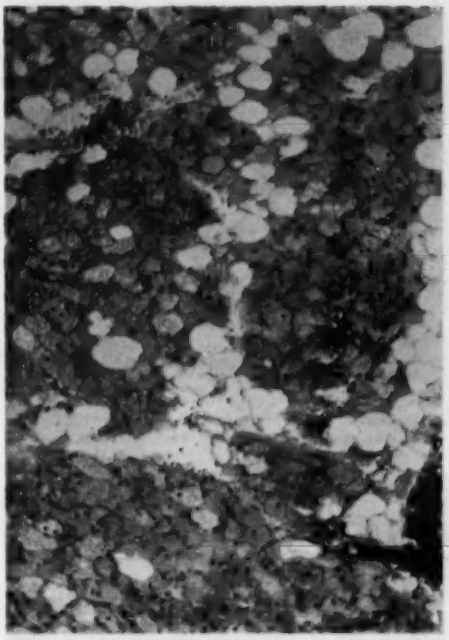
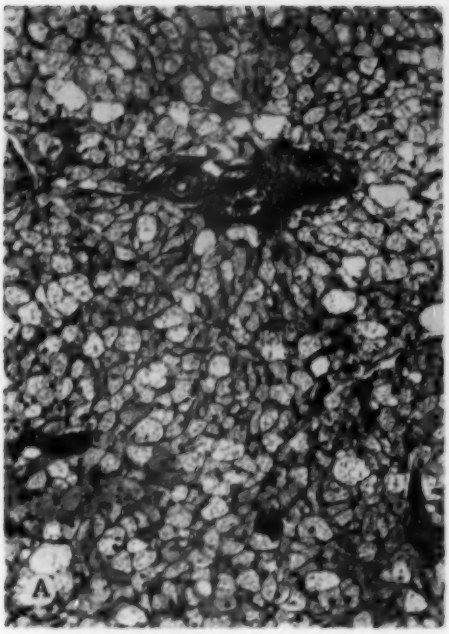


Fig. 1.—Hibernating fat in close approximation to a pheochromocytoma of the left adrenal gland weighing 7 gm. from a 52-year-old woman. Reduced to 76% of mag. × 100.

Submitted for publication Sept. 19, 1956.

From the Squier Urological Clinic and Presbyterian Hospital, Columbia University College of Physicians and Surgeons.



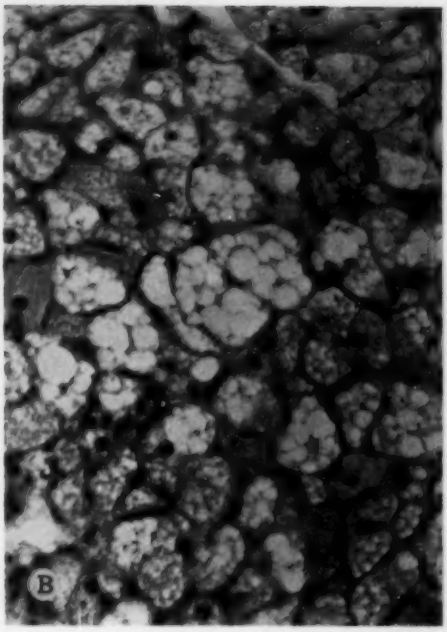




Fig. 2.—*A*, hibernating fat close to a pheochromocytoma of the right adrenal gland weighing 65 gm. from a woman, age 30, with sustained hypertension. Note foam cells. Reduced to 76% of mag. × 100. *B*, same case, higher magnification. Reduced to 76% of mag. × 250.

Fig. 3.—Hibernating fat showing division into lobules. Pheochromocytoma of right adrenal gland weighing 60 gm. from a 28-year-old man with sustained hypertension.

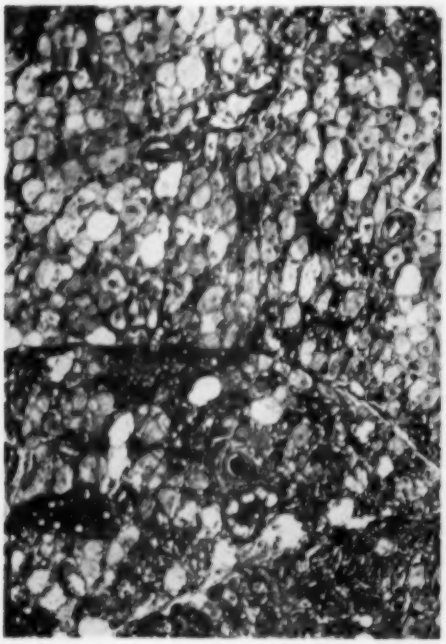
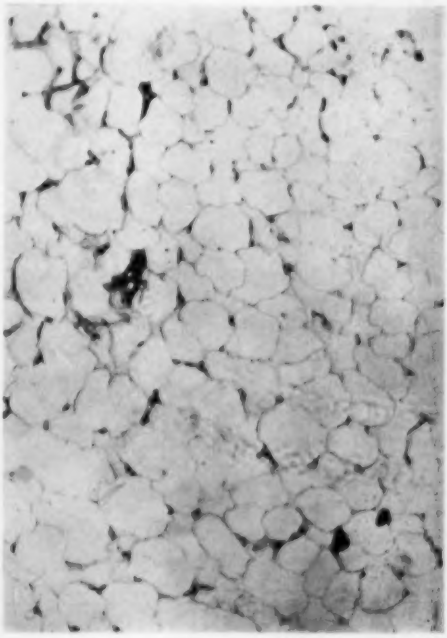


Fig. 4.—Biopsy specimen of subcutaneous fat from same case as that shown in Figure 2. Note adult fat.



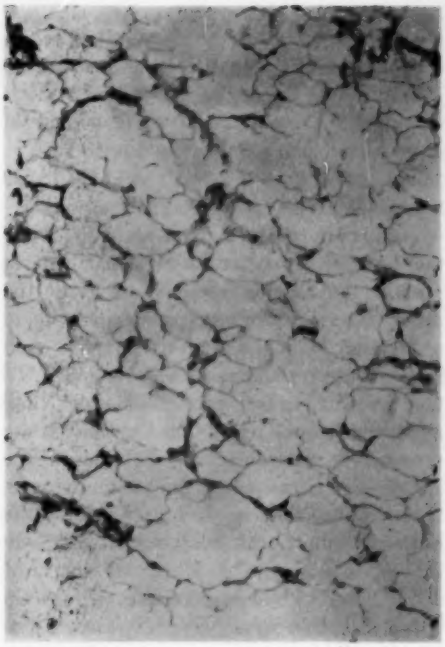


Fig. 5.—Biopsy specimen of perirenal fat from same case as that shown in Figure 2. Note adult fat.

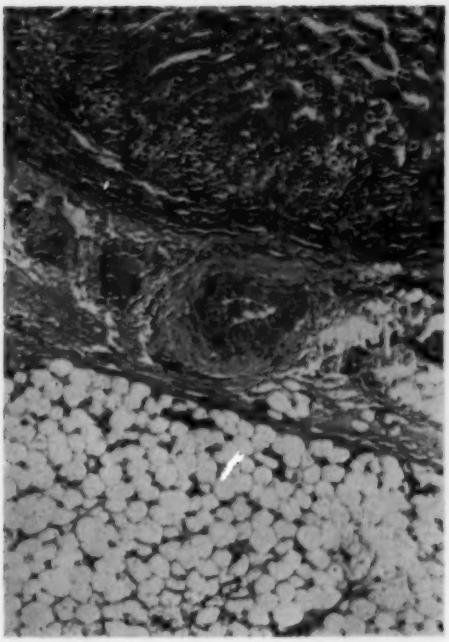


Fig. 6.—Periadrenal adult fat from a girl, age 2 years, with cortical hyperplasia causing adrenogenital syndrome.

Fig. 7.—Adult fat surrounding cortical adenoma from a boy, age 7 years, with adrenogenital syndrome.

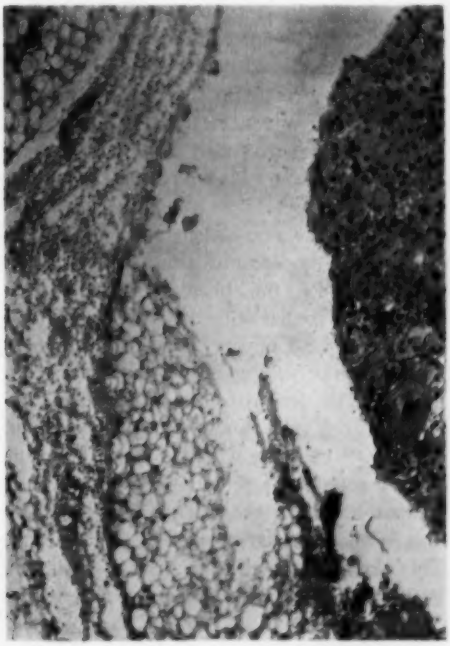


Fig. 8.—Adult fat in association with cortical adenoma from a woman, age 28, with Cushing's syndrome.

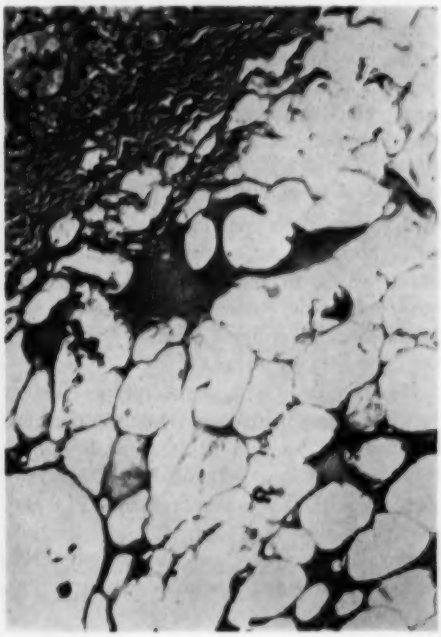


Fig. 9.—Adult fat in association with a sympathicoblastoma of the adrenal medulla in a 4-year-old boy.

### White Fat Versus Brown Fat

There are two types of fat: one, the commoner, which is characteristic of humans, is known as the "yellowish" or "white" or "adult" fat and is composed of large cells (average, 40μ) filled with single large vacuoles capped by arcuate nuclei, which give the cells a signet-ring appearance. The other is the "brown" fat (also called hibernating, primitive, multilocular, glandular, embryonal fat) which is the normal characteristic of the hibernating glands of many mammals, particularly those with seasonal dormant cycles (marmot, bat, hedgehog, and other rodents [1,14]).

Hibernating fat cells occur in clusters forming brownish-colored lobules that are encapsulated by thin fibrous sheaths. The cells are round or polygonal with centrally placed nuclei. They average about 25μ and, though smaller than adult fat cells, possess a considerably richer vascular supply. Numerous lipid-filled vacuoles are present, which give the cells their multilocular or "foamy" appearance.[1] The amount of unsaponifiable fat was found to be higher in hibernomas than in lipomas.[8]

There are three principal zones of distribution of brown fat in hibernating animals: interscapular, thoracic, and abdominal. In the last area the fat is seen particularly around the adrenal glands and about the renal vessels. It occurs occasionally in these areas even in nonhibernating animals, such as the white rat and mouse. In monkeys,[1,13] too, brown fat can occur. The principal sites are in the axillae and neck, in the thoracic paravertebral region, and in the soft tissues surrounding the adrenal glands, between the kidneys, and in front of the aorta. A similar distribution has been seen in human embryos.[5,9] Rarely a tumor of foam cells, a "hibernoma," can form from these cell collections.[4,6,10,15,17] Brown fat has also been reported in a case with Addison's disease and diabetes [11] and in cretins.[16,18]

### Comment

The frequent association of hibernating fat with pheochromocytoma, as observed in our series of 16 out of 18 cases since 1950, may have been fortuitous. Brown fat may normally be present in the periadrenal area in greater frequency than is suspected. This brown fat may also be associated with lesions of the adrenal gland other than pheochromocytoma and was missed in our series because it was not looked for. If, on the other hand, the accumulation of hibernating fat is related to the chromaffin tumors, then we are confronted with the problem of possible cause and effect. It is of interest to note that hibernating fat contains increased quantities of demonstrable phospholipid, ascorbic acid, alkaline phosphatase, and esterase.[1,3] Gross and Wood [8] noted significant amounts of birefringent crystalline material in Sudan-stained sections of hibernomas on examination with polarized light. This doubly refractile material was similar to that described by Ayres et al.[2] in the

adrenal cortex. Cramer [7] regarded the presence of similar anisotropic material in the brown fat and adrenal cortex of the white rat and mouse as a significant point of similarity. He noted also that the brown fat became more glandular in appearance and more vascular in animals kept on a vitamin-deficient diet.[7,12] Selye and Timiras [15] discussed the participation of brown fat tissue in the alarm reaction. There seemed to be some relationship between brown fat and the adrenal gland; the discharge of cortical lipids paralleled the loss of sudanophilic material from the hibernating fat. Selye and Timiras found that the stresses and strains induced by the alarm status associated with the chromaffin tumors may be additional factors in stimulating metabolism of brown fat.

If the association of hibernating fat with pheochromocytoma is constant and exclusive, then finding such fat adjacent to an adrenal tumor, or of its remnants, or of a cyst at operation or autopsy, may aid in differential diagnosis in clinically and/or histologically doubtful cases. Laqueur and Harrison [11] reported finding glandular fat surrounding the extensively destroyed adrenals of an 18-year-old boy who had "diabetes" since the age of 12. The cortex was absent in most places, and the medulla contained sheets of collagenous tissue imprisoning nodules of large cells with giant nuclei and cytoplasmic pigment granules. Owing to a hemorrhagic tendency, pheochromocytomas often undergo necrosis and fibrosis, and we have often seen changes in these tumors which were similar to those described by the authors. The possibility of a bilateral pheochromocytoma that underwent extensive destruction might be considered in this case.

## Summary and Conclusions

Hibernating fat (also called brown, embryonal, or glandular fat) was found in varying amounts in the pericapsular region in 16 out of 18 pheochromocytomas removed over a three-year period from 1953 to 1956.

Only adult fat was found adjacent to adrenal tissue or adenomas or carcinomas removed from patients with adrenogenital or Cushing's disease, from a patient suffering from sympathicoblastoma, and from 3 patients with renal carcinoma.

Biopsy of subcutaneous, retroperitoneal, perirenal fat in three patients with pheochromocytoma embedded in hibernating fat showed only adult adipose tissue.

Adult (white fat) and brown fat (hibernating) were compared as to occurrence, site, and histology.

Hibernating fat is more vascular than the adult variety and has been shown to undergo marked edema and hyperemia, with discharge of sudanophilic lipids during exposure to stress. A possible relationship may exist between the added stress (alarm reaction) of pheochromocytoma and the development and metabolism of glandular (hibernating) fat.

Dept. of Urology, Columbia University College of Physicians and Surgeons (32).

## REFERENCES

1. Aronson, S. M., and Shwartzman, G.: Histopathology of Brown Fat in Experimental Poliomyelitis, Am. J. Path. 32:315-333, 1956.

2. Ayres, W. W.; Firminger, H. I., and Hamilton, P. K.: Birefringent and Sudanophilic Lipoids in the Adrenal Cortex in Disease and Sudden Death, Mil. Surgeon 109:503-517, 1951.

3. Bacchus, H.: Cytochemical Study of the Adrenal Cortex of the Rat Under Salt Stresses, Am. J. Physiol. 163:326-331, 1950.

4. Bonnel, F.: Tumeur du creux de l'aisselle, Bull. et mém. Soc. Anat. Paris 79:110-112, 1914.

5. Bonnot, E.: The Intercapsular Gland, J. Anat. 43:43-58, 1908.

6. Brines, O. A., and Johnson, M. H.: Hibernoma, a Special Fatty Tumor: Report of a Case, Am. J. Path. 25:467-479, 1949.

7. Cramer, W.: On Glandular Adipose Tissue, and Its Relation to Other Endocrine Organs and to the Vitamine Problem, Brit. J. Exper. Path. 1:184-196, 1920.

8. Gross, S., and Wood, C.: Hibernoma, Cancer 6:159-163, 1953.

9. Hatai, S.: On the Presence in Human Embryos of Interscapular Gland Corresponding to the So-Called Hibernating Gland of Lower Mammals, Anat. Anz. 21:369-373, 1902.

10. Kittle, C. F.; Boley, J. O., and Schafer, P. W.: Resection of an Intrathoracic "Hibernoma," J. Thoracic Surg. 19:830-836, 1950.

11. Laqueur, G. L., and Harrison, M. B.: Glandular Adipose Tissue Associated with Cytotoxic Suprarenal Contraction and Diabetes Mellitus, Am. J. Path. 27:231-245, 1951.

12. Menschik, Z.: Histochemical Study of Developing Adipose Tissue in Guinea-Pigs Under the Influence of Vitamin E, Anat. Rec. 109:325, 1951.

13. Merkel, H.: Über ein Pseudolipom der Mamma. (Eigenartiger Fettzellentumor). Beitr. path. Anat. u. allg. Path. 39:152-157, 1906.

14. Rasmussen, A. T.: The So-Called Hibernating Gland, J. Morphol. 38:147-205, 1923.

15. Selye, H., and Timiras, P. S.: Participation of "Brown Fat" Tissue in the Alarm Reaction, Nature, London 25:10, 467, 1949.

16. Shattock, S. G.: The Fat Masses in the Necks of Cretins, Proc. Roy. Soc. Med. 2:207-226, 252, 1909.

17. Sutherland, J. C.; Callahan, W. P., Jr., and Campbell, G. L.: Hibernoma: A Tumor of Brown Fat, Cancer 5:364-368, 1952.

18. Wells, H. G.: Adipose Tissue, a Neglected Subject, J. A. M. A. 114:2177-2183; 2284-2289, 1940.

# Some Observations on the Histochemical Reactions of Amyloid

G. M. WINDRUM, M.B., D.Phil., and H. KRAMER, B.M., D.Phil., Brisbane, Queensland, Australia

A considerable amount of work has been reported in recent years on the histochemical reactions of the substances which appear in the connective tissues in various pathological conditions. Many of these can be grouped under the heading of "hyaline substances" and the conditions in which they occur as hyaline degenerations, a term first used by von Recklinghausen (1883). Hyaline substances form a heterogeneous group which have in common the property of appearing translucent, refractile, and homogeneous in tissue sections. The term "hyaline" denotes the physical appearance rather than a characteristic chemical composition. It is widely applied to describe the changes in such apparently unrelated conditions as the arterial subendothelial thickening of arteriosclerosis, old scar tissue, the stromal changes in some tumors, and the material of the corpora amylacea of brain and prostate. Though most commonly applied to extracellular substances, the term is also sometimes used to describe intracellular conditions, as in Zenker's degeneration of voluntary muscle.

Amyloid is one variety of hyaline substance, but it is given a special name because of its more characteristic anatomical situation and because it has a number of more or less constant staining characteristics. The substance was called amyloid by Virchow (1854) because it stained somewhat like starch with iodine.

Four types of amyloidosis are now distinguished: the primary systemic form; the systemic form associated with multiple myeloma; the local, or tumor, variety, and the secondary form associated with chronic diseases. The last is the commonest, and the deposits are found in the spleen, liver, kidney, and adrenal, where they appear histologically as homogeneous acidophilic deposits in the characteristic extraendothelial situation. The other varieties may affect connective tissues anywhere in the body but generally do not involve the structures affected in the secondary type.

The chemical composition of most hyaline substances is not known, but that of amyloid has been investigated on a number of occasions.

In 1894 Oddi isolated reducing substances and sulfate from amyloid tissue and suggested that amyloid contained a carbohydrate fraction. Krawkow (1898) isolated a protein fraction from amyloid-bearing organs, and also a fraction which had some of the properties of chondroitin-sulfuric acid. On the other hand, Hansen (1908) could find no evidence of a carbohydrate component in the amyloid he isolated from splenic tissue. Eppinger (1922) analyzed an amyloid tumor from the liver of a young patient who had no disease which could be held responsible for the development of the amyloidosis, and found that the tumor had no qualitatively detectable carbohydrate. More recent investigations on secondary amyloidosis, those of Hass and Schulz (1940) and Hass (1942), have indicated that in the amyloid deposits occurring in the livers of humans dying of chronic pulmonary tuberculosis there are at least two protein fractions, and also a polysaccharide of the chondroitin-sulfuric acid type. Hass (1942) found that,

Submitted for publication Sept. 8, 1956.

From the Department of Pathology, Brisbane Hospital, and the Cancer Research Unit, Prince of Wales Hospital, Randwick, New South Wales, Australia.

though this polysaccharide was constantly present in such deposits, it was present in variable amounts, ranging from 0.5% to 1.5% of the total amyloid material.

Amyloid is now generally considered to be a glycoprotein in which a mucopolysaccharide is attached to a globulin, and to be the result of a disturbance of protein synthesis in mesenchymal cells (Teilum, 1952). The precise factors which govern the formation and deposition of amyloid are unknown, but there are reasons for believing that in vivo interactions between antigens and immune bodies are concerned. Many regard amyloid as an antigen-antibody precipitate, and Teilum (1952) has shown that cortisone and ascorbic acid may affect the deposition of amyloid, just as these substances may affect other aspects of connective tissue function.

In spite of considerable evidence for the presence of sulfated polysaccharide, deposits in secondary amyloidosis do not exhibit true metachromasia (Kramer and Windrum, 1955). Cornil (1875) showed that staining of tissue sections containing amyloid deposits with methyl violet resulted in a dissociation of the dye, so that the amyloid deposits stained a red-violet color and most other tissue elements a blue-violet color. This was not described as a metachromatic phenomenon by Cornil (1875); indeed, the term "metachromasia" was not used at that time, being first introduced by Ehrlich (1877). However, numerous authors, including Gomori (1952), describe methods for the demonstration of amyloid by its "metachromatic" properties, by using such dyes as methyl violet and crystal violet. It can be shown by paper chromatograms that these dyes are not single substances but mixtures of differently colored dyes. Staining of tissues with such mixtures does not therefore necessarily represent a true metachromatic technique, but may be only a selective staining by one of the colored components of the dye mixture.

The staining properties and histoenzymic reactions of deposits in secondary amyloidosis have been compared with those of collagen and old scar tissue. These three materials occur extracellularly in connective tissue, and their production is apparently related to the activity of mesenchymal cells. Therefore, it seemed a useful study to compare their reactions. The ovary was chosen as a convenient site for the demonstration of collagen and scar tissue, collagen being abundant in the tunica albuginea and scar tissue of different ages being represented in the same organ by corpora albicantia.

## Materials and Methods

*A. Histological Material.*—Blocks of tissue from ovaries of patients without amyloidosis and from amyloid-bearing livers and kidneys of patients dying of chronic pulmonary tuberculosis were used. They were fixed in graded alcohols and embedded in paraffin. Formol fixation was avoided, as formalin-stabilized collagen resists the action of collagenase (Robb-Smith, 1953). Sections were cut at 6μ-8μ and mounted on glass slides in pairs, one section near the end of the slide, and the other nearer the middle. Frozen sections from tissues fixed in formol-saline were used to determine the presence of lipid material.

*B. Histoenzymic Methods.*—The sections were deparaffinized and hydrated in the usual way before incubation in the enzyme solutions in Coplin jars at 37 C. These jars were slightly less than half full, so that only one section was covered by the enzyme solution and the other served as an undigested control. Other control sections were incubated in the appropriate buffer solutions and in solutions of the enzymes inactivated by heating.

The following enzyme preparations were used:

1. Pepsin (Evans Medical Supplies, Ltd., London): This was prepared as follows: pepsin, 500 mg.; concentrated hydrochloric acid, 0.3 ml.; isotonic (0.85%) saline solution, 100 ml. Incubation was performed for 3, 6, 12, and 24 hours.
2. Trypsin (British Drug Houses): 200 mg. per 100 cc. in a phosphate buffer at pH 8 for 12 hours.
3. Testicular hyaluronidase (Rondase, Evans Medical Supplies, Ltd., London): 100 mg. per 100 cc. in a borate buffer at pH 6.8 for 12 hours.
4. Pectozyme (Norman Evans and Rais, Ltd., Manchester, England: 2% in an acetate buffer pH 5 for 60 hours.
5. Diastase (British Drug Houses): 0.1% in borate buffer at pH 8 for four hours.

6. Collagenase (Clostridium welchii): 25 mg. per 100 cc. in a borate buffer at pH 6.8 for 12 and 24 hours. This collagenase preparation had the following characteristics: 39κ equivalents (Azocoll) per milligram; 39κ equivalents (collagen paper) per milligram; 36κ equivalents (muscle) per milligram; hyaluronidase activity, 1 unit per milligram.

The κ equivalent is the unit of standardization of C. welchii collagenase, the term κ toxin being introduced by Oakley, Warrack, and van Heyningen (1946) to designate this antigenically distinct toxin present in C. welchii Type A filtrates.

Azocoll equivalents are not always a good indication of the true collagenase activity of a preparation, as the collagen in Azocoll may be partially denaturated (Oakley, Warrack, and Warren, 1948); the collagen paper equivalents are a more reliable guide. In this case, the enzyme solution used had an activity of approximately 975κ equivalents (collagen paper) per 100 ml. of buffer. To neutralize the effect of the small amount of hyaluronidase present, a specific anti-C.-welchii hyaluronidase serum was used. This contained 1000 units of antihyaluronidase activity per milliliter and only 5 units of anticollagenase activity per milliliter. Double the amount of antihyaluronidase serum needed to ensure neutralization was used, and this amount had a negligible anticollagenase effect. The collagenase preparation was said to have no lecithinase activity and only very slight deoxyribonuclease activity.

*C. Staining Reactions.*—Undigested sections, digested sections, and the appropriate controls were stained with azure A to demonstrate metachromasia, as described by Kramer and Windrum (1955). The PAS reaction (McManus, 1946 and Hotchkiss, 1948), the sulfation-metachromasia reaction (Kramer and Windrum, 1953; 1954), and the hematoxylin-eosin and Van Gieson stains were also used.

## Results

The accompanying Table shows the results of the staining reactions before and after the enzymic digestions.

Some of the trypsin-digested slides were difficult to stain, and many of the sections digested in pepsin for the longer periods were lost during the incubation and subsequent washing. However, sufficient sections survived to enable the effects on the staining reactions to be assessed.

From the Table it can be seen that normal collagen and old scar tissue had essentially similar staining reactions and reacted similarly to enzymic digestions. The only significant differences noted were that the staining of scar tissue was usually less intense than that of collagen, and that in frozen sections some sudanophilic material was occasionally associated with the scar tissue, but not with the collagen. The digestion of scar tissue by pepsin and collagenase was, in general, more rapid than that of collagen. However, there was some variation in the rate of digestion of scar

*Staining Reactions Before and After Enzymic Digestion* *

| Treatment | Material | Azure A | PAS | Sulfated Met. | H & E | Van Gieson |
|---|---|---|---|---|---|---|
| Untreated | Amyloid | Blue | Moderate pink | Red | Bright pink | Orange |
| | Collagen | Blue | Faint pink | Blue | Pink | Red |
| | Scar | Blue | Faint pink | Blue | Pink | Red |
| Pepsin | Amyloid | Red | Faint pink | Red | Pink | Faint orange |
| | Collagen | D | D | D | D | D |
| | Scar | D | D | D | D | D |
| Trypsin | Amyloid | Blue | Faint pink | Red | Pink | Faint orange |
| | Collagen | Blue | Faint pink | Blue | Pink | Red |
| | Scar | Blue | Faint pink | Blue | Pink | Red |
| Hyaluronidase (Hyalase) | Amyloid | Blue | Moderate pink | Red | Bright pink | Orange |
| | Collagen | Blue | Faint pink | Blue | Pink | Red |
| | Scar | Blue | Faint pink | Blue | Pink | Red |
| Pectozyme | Amyloid | Blue | Faint pink | Faint red | Pink | Orange |
| | Collagen | Blue | Faint pink | Blue | Pink | Red |
| | Scar | Blue | Faint pink | Blue | Pink | Red |
| Diastase | Amyloid | Blue | Moderate pink | Red | Bright pink | Orange |
| | Collagen | Blue | Faint pink | Blue | Pink | Red |
| | Scar | Blue | Faint pink | Blue | Pink | Red |
| Collagenase | Amyloid | Blue | Moderate pink | Red | Bright pink | Orange |
| | Collagen | D | D | D | D | D |
| | Scar | D | D | D | D | D |

*D=completely digested.

tissue. Some of the old corpora albicantia seemed to resist peptic and collagenase digestions for periods up to 12 hours, by which time most of the collagen of the tunica albuginea and stroma, reticulin, and less dense scar tissue had been completely removed. After 24 hours, however, all this dense material had been digested as well.

Amyloid, on the other hand, showed quite different reactions; it was more eosinophilic, failed to show fuchsinophilia with Van Gieson's stain, gave a moderate PAS reaction and stained a striking red with the sulfation-metachromasia reaction. The frozen sections showed no sudanophilia.

In contrast to its effect on collagen and old scar tissue, the collagenase preparation had no effect on the staining of the amyloid deposits. Peptic digestion showed that the amyloid was relatively more resistant to this proteolytic enzyme than was either collagen or scar tissue. Incubation of sections containing amyloid in pepsin for three to six hours changed the staining of the amyloid from orthochromatic to metachromatic with toluidine blue or azure A. Further digestion, up to 24 hours, reduced this metachromatic coloration progressively, so that the remnants of the amyloid left showed only a very faint metachromatic color. The emergence of metachromasia was accompanied by a reduction in the intensity of PAS staining.

Tryptic digestion of the amyloid resulted in a pronounced decrease in the intensity of staining by the PAS reaction. It did not alter the sulfation-metachromasia reaction, and did not result in metachromatic staining of the residual material, as did the peptic digestion.

Digestion with hyaluronidase or diastase did not significantly alter the staining characteristics of amyloid, collagen, or the old scar tissue. Prolonged digestion with Pectozyme reduced the PAS reaction and the sulfation-metachromasia reaction of amyloid, but did not alter the staining of collagen or scar tissue.

Some further experiments were done by digesting sections containing amyloid for six hours in pepsin and then further digesting them in hyaluronidase. The peptic digestion resulted in the appearance of the metachromatic staining in the amyloid deposits, as noted above, and subsequent hyaluronidase digestion altered this to an orthochromatic staining in some areas, but not in all. The exposure of slides, partially digested by pepsin, to 30% potassium chloride with 1% potassium carbonate (Einbinder and Schubert, 1950) resulted in a loss of all the metachromatic material after four days.

## Comment

The results obtained when amyloid is partially digested with pepsin suggest that a sulfated polysaccharide is actually present in the material, but is normally prevented from exerting its metachromatic potency because the sulfate radicals are masked by combination with protein. This finding was reported previously (Kramer and Windrum, 1955) in a discussion on the normal metachromatic staining reaction, and the present paper presents an elaboration of that work. The mucopolysaccharide is probably of chondroitin-sulfuric-acid type, but some of this is not removable by hyaluronidase even after the initial peptic digestion. It is interesting to note that if amyloid is exposed to testicular hyaluronidase, no effect on its subsequent staining reactions is observed. This suggests that the mucopolysaccharide is initially very closely bound to the protein constituents. Follis (1951) has reported that partial peptic digestion of skin collagen will render it metachromatic by unmasking previously bound sulfated mucopolysaccharide, but this has not been confirmed on skin sections or on the collagen or scar tissue seen in the ovaries examined here. The unmasking of metachromasia in amyloid deposits by peptic digestion was accompanied by a loss of PAS staining. This suggests that there may actually be two carbohydrate components in amyloid; one, responsible for the PAS reaction, is lost after both peptic and tryptic digestion; the other, which is responsible for the metachromasia, is un-

masked by peptic, but not by tryptic, digestion. The main protein component of amyloid is usually considered to be a globulin (Teilum, 1952), and globulins may contain carbohydrate and be PAS-positive (Pearse, 1953). This might well be the component that is responsible for the PAS staining and which is readily removed by the proteolytic digestions.

Altshuler and Angevine (1951), after an extensive study of the role of mucopolysaccharides in serous inflammation, suggested that these substances may be precipitated with protein in the small space that normally exists between capillary and cell membranes, the so-called hematoparenchymal space. They suggested that acidic mucopolysaccharides could unite with basic proteins to form complexes in these situations. It is known that sulfated mucopolysaccharides may actually bind proteins, and French and Benditt (1953) have stressed that proteins may modify the reactions between dyes and polysaccharides by competing with the dye for the available carbohydrate groups, thus leading to masking of the polysaccharide. French and Benditt (1953) showed that treatment of cartilage sections with solutions of proteins could completely block the metachromasia of such cartilage sections. It would seem that a similar effect may be produced in amyloid by protein binding, and that the peptic digestion removes enough of this protein to allow the mucopolysaccharide to exhibit its previously masked metachromatic potency.

Although carbohydrate can be demonstrated in amyloid material, the hyaline material of old scar tissue shows no histochemical evidence for the presence of carbohydrates, nor can any be unmasked by enzymic digestion. In the early stages of development of scar tissue abundant extracellular metachromatic material can be seen. This is gradually lost as the scar matures, and the material left has all the tinctorial and enzymic reactions of collagen, to which a small quantity of sudanophilic material may sometimes be added. On the other hand, mucopolysaccharide may play an essential part in the deposition of amyloid, but, instead of being lost later, may persist in the mature material.

It is of interest to record that a potent collagen-digesting enzyme has no effect on deposits in secondary amyloidosis. Robb-Smith (1945) was the first to report the use of distinct collagenase preparations for histoenzymic studies. He used a collagenase present in C. welchii Type A toxin, and was able to show that both histological reticulin and collagen were readily removed from sections of tissue by these means.

The literature on the use of collagenase is confused, due mainly to the fact that inadequate preparations of limited potency have been used; often denatured collagen or gelatin has been used to demonstrate the enzyme potency, instead of native collagen. Robb-Smith (1953) has reviewed the development of the use of collagenase preparations and has drawn attention to such failure to ensure the potency of the preparations.

In this work, a lyophilized preparation of C. welchii collagenase was used. Preliminary histoenzymic studies showed that this preparation was capable of removing reticulin, collagen, and the metachromatic ground substance of arterial walls. Further investigations confirmed that the preparation had some hyaluronidase, as well as collagenase, activity. An antihyaluronidase serum was therefore added to the collagenase solutions used, and this reduced, but did not entirely prevent, the removal of metachromatic ground substance. It may be that the collagenase removed this material by attacking the protein with which it is associated, rather than by hydrolyzing the sulfated polysaccharide itself. Nevertheless, the enzyme preparation had no effect on the reactions of amyloid, suggesting that the protein component is not collagen.

These studies, in which the histochemical and histoenzymic reactions of amyloid and connective tissue are compared, indicate how such analysis may help to subdivide and

characterize pathological substances which are biochemically ill-defined. The technique might usefully be applied to the different varieties of amyloid to define their differences and similarities more closely.

## Summary

1. Partial peptic digestion of the deposits in secondary amyloidosis will change the staining reaction from orthochromatic to metachromatic with toluidine blue or azure A.

2. Most of this metachromatic material may be removed by subsequent digestion with testicular hyaluronidase, suggesting the presence of a polysaccharide of the chondroitin-sulfuric-acid type.

3. A potent collagen-digesting enzyme has no effect on the staining reactions of amyloid, indicating that the protein moiety is not collagen.

Most of this work was carried out at the Radcliffe Infirmary, Oxford, England. Prof. C. L. Oakley, late of Wellcome Physiological Research Laboratories, Beckenham, Kent, made the preparations of collagenase and C. welchii antihyaluronidase serum.

Brisbane Hospital, Herston Rd.

## BIBLIOGRAPHY

Altshuler, C. H., and Angevine, D. M.: Acid Mucopolysaccharide in Degenerative Disease of Connective Tissue, with Special Reference to Serous Inflammation, Am. J. Path. 27:141-156, 1951.

Cornil, V.: Sur la dissociation du violet de methylaniline et sa séparation en de couleurs sous l'influence de certains tissus normaux et pathologiques, en particulier par les tissus en dégénérescence amyloide, Compt. rend. Acad. Sc. 80:1288-1291, 1875.

Ehrlich, P.: Beiträge zur Kenntniss der Anilinfärbungen und ihrer Verwendung in der mikroskopischen Technik, Arch. mikr. Anat. 13:263-277, 1877.

Einbinder, J., and Schubert, M.: Separation of Chondroitin Sulfate from Cartilage, J. Biol. Chem. 185:725-730, 1950.

Eppinger, H.: Zur Chemie der amyloiden Entartung, Biochem. Ztschr. 127:107-111, 1922.

Follis, R. H., Jr.: Effect of Proteolytic Enzymes and Fixation on Metachromasia of Skin Collagen, Proc. Soc. Exper. Biol. & Med. 76:272-273, 1951.

French, J. E., and Benditt, E. J.: Histochemistry of Connective Tissue: II. Effect of Proteins on the Selective Staining of Mucopolysaccharides by Basic Dyes, J. Histochem. 1:321-325, 1953.

Gomori, G.: Microscopic Histochemistry: Principles and Practice, Chicago, University of Chicago Press, 1952, p. 76.

Hansen, O.: Ein Beitrag zur Chemie der amyloiden Entartung, Biochem. Ztschr. 13:185-198, 1908.

Hass, G.: Studies of Amyloid: II. The Isolation of a Polysaccharide from Amyloid-Bearing Tissues, Arch. Path. 34:92-105, 1942.

———and Schulz, R. Z.: Amyloid: I. Methods of Isolating Amyloid from Other Tissue Elements, Arch. Path. 30:240-259, 1940.

Hotchkiss, R. D.: Microchemical Reaction Resulting in the Staining of Polysaccharide Structures in Fixed Tissue Preparations, Arch. Biochem. 16:131-141, 1948.

Kramer, H., and Windrum, G. M.: Metachromasia After Treating Tissue Sections with Sulphuric Acid, J. Clin. Path. 6:239-240, 1953.

Sulphation Techniques in Histochemistry with Special Reference to Metachromasia, J. Histochem. 2:196-208, 1954.

The Metachromatic Staining Reaction, J. Histochem. 3:227-237, 1955.

Krawkow, N. P.: Beiträge zur Chemie der Amyloidentartung, Arch. exper. Path. u. Pharmakol. 40:195-220, 1898.

McManus, J. F. A.: Histological Demonstration of Mucin After Periodic Acid, Nature, London 158:202, 1946.

Oakley, C. L.; Warrack, G. H., and van Heyningen, W. E.: The Collagenase (Kappa Toxin) of Cl. Welchii Type A., J. Path. & Bact. 58:229-235, 1946.

———Warrack, G. H., and Warren, M. E.: The Kappa and Lambda Antigens of Clostridium Welchii, J. Path. & Bact. 60:495-503, 1948.

Oddi, R.: Über das Vorkommen von Chondroïtinschwefelsäure in der Amyloidleber, Arch. exper. Path. u. Pharmakol. 33:376-388, 1894.

Pearse, A. G. E.: Histochemistry: Theoretical and Applied, London, J. & A. Churchill, Ltd., 1953.

Recklinghausen, F., von: Handbuch der allgemeinen Pathologie des Krieslaufs und der Ernährung, Stuttgart, F. Enke, 1883.

Robb-Smith, A. H. T.: Tissue Changes Induced by Cl. welchii Type A Filtrates, Lancet 2:362-368, 1945.

Nature and Structure of Collagen, London, Butterworth & Company (Publishers), Ltd., 1953, pp. 14-26.

Teilum, G.: Cortisone-Ascorbic Acid Interaction and the Pathogenesis of Amyloidosis: Mechanism of Action of Cortisone on Mesenchymal Tissue, Ann. Rheumat. Dis. 11:119-136, 1952.

Virchow, R.: Zur Cellulose-Frage, Arch. path. Anat. 8:416-426, 1854.

# Duplication of Large Intestine Associated with Multiple Malformations

FREDERICK P. BORNSTEIN, M.D., El Paso, Texas

Duplications of part of the intestinal tract represent a rather uncommon malformation. Some confusion exists because certain cystic, saccular structures have been mistakenly called duplications of the intestine.[1] However, a number of genuine duplications have been reported in the literature, and the subject has been reviewed and summarized by Ravitch,[2] who collected 21 cases from the literature. It is noticeable that none of these cases show exactly identical malformations. This additional case is reported because, again, the structure of the malformation differs in some important parts from the previously reported ones. Also, as is rather common, there were malformations in other organ systems, and these are incompletely reported in many of the former cases.

## Report of a Case

The patient, a girl, was born on March 8, 1956, and admitted to the El Paso General Hospital the next day because of the presence of multiple malformations. The family history was not remarkable. The mother was a sextigravida, quintipara, and delivery was uneventful.

Physical examination revealed a newborn girl with congenital spina bifida, a meningocele, a congenital defect in the anterior abdominal wall, and a malformation of the vulva. The descriptions available state that feces and urine were discharged from the vulva and that no perineum was found. X-ray examination of the pelvis was done on the day of admission, and the findings were as follows (Dr. V. Ravel):

"Survey film of the abdomen reveals moderate amount of gas in the small intestine. There is marked separation of the pubic bones, consistent with an extrophy of the bladder. There is a congenital anomaly of the sacrum, suggestive of rachischisis.

"Conclusions: Congenital anomalies of the pelvis."

The patient was hospitalized, and during her stay in the hospital she developed a progressive hydrocephalus and died on July 9, 1956. The following observations were made at autopsy.

*External Description.*—There is extensive hydrocephalus. A meningocele, oval in shape, which measures about 6 cm. in greatest diameter, is located in the thoracic region of the spine. The chest is symmetrically shaped; the abdomen is flat. The umbilicus is missing. From the umbilical region downward there is a circular area, measuring about 5 cm. in greatest diameter, where the abdominal wall is flattened out and has a light-brown discoloration.

The external genitals are deformed. There is a genital fold running in the midline, producing atypical labia majora. Five foramina are present in the groove formed by the labia majora. In the superior region of the groove a small clitoris is visible. Slightly beneath and lateral to the clitoris two small foramina are present. Farther down an oval foramen is present. Beneath this opening two foramina are present, one somewhat more to the left than the other and superior to it. Feces can be expressed from these two foramina (Fig. 1). After the skin has been reflected, it is seen

Fig. 1.—The external genitals. Visible are the clitoris, the vaginal opening, and the two ani. The urethrae are covered by the genital fold.

Submitted for Publication Dec. 3, 1956.

Department of Pathology, El Paso General Hospital.

Fig. 2.—Posterior aspect showing bifurcation of terminal ileum, two appendices, and the duplicated colon.

that the pelvis is wide open and that the symphysis pubis is absent.

*Body Organs.*—The chest organs show the presence of bronchopneumonia.

Abdomen: The intestine is partially malrotated. After lifting the intestine the following abnormalities are found: A duplication starts with a bifurcation of the terminal ileum about 6 cm. above the ileocecal valve. The entire large bowel, including the appendix, is duplicated (Fig. 2). These structures are partially freely movable and partially joined together by a thin membrane. Each of the large bowels terminates separately into the inferior foramina located in the genital groove.

The right kidney and ureter are found in the usual place. The left kidney is found deep in the pelvis, fixed against the wall with the renal pelvis markedly dilated. Each of the ureters leads into a separate urinary bladder, with a separate urethra. The urethral openings represent the superior foramina found in the genital fold. The third (middle) foramen represents the vagina, which can be traced to the single uterus, with normal tubes and ovaries. The remainder of the examination is not remarkable.

In summary, then, we are dealing with five foramina. Each of the two superior foramina connects with a separate urinary bladder. Each of the inferior foramina connects with a separate rectum. The central foramen represents the vagina. All these foramina are located in a groove between the labia majora and below the clitoris. The perineum is absent. In addition, no proper sphincter ani is present in either rectum, but both rectal openings are functioning and are capable of discharging feces.

*Anatomical Diagnosis.*—(1) Multiple malformations—hydrocephalus, meningocele, duplication of large intestine and lower urinary tract, absence of symphysis pubis; (2) bronchopneumonia.

## Comment

Each of the various lesions found in this case has been described in the literature, although none in exactly the same combination. The most unusual feature here is the observation that the duplication was limited to the intestinal and urinary tracts and that no duplication of the genital tract had occurred. It is also unusual that each rectum had a functioning opening. In most of the cases described, one of the duplicated intestines ended in a blind sac, producing distention with secondary symptoms, whereas in the present case both intestinal tracts were functioning normally except that neither had a competent sphincter.

Concerning the etiology of this lesion, a number of speculations have been produced previously, to which no addition need be made.

## Summary

A case is reported of duplication of the large bowel, including the appendix, and lower urinary tract associated with malformations of the bony and central nervous systems. This is the 22d case so described in the literature.

Providence Memorial Hosp., 2001 N. Oregon St.

## REFERENCES

1. Potter, E. L.: Pathology of the Fetus and the Newborn, Chicago, The Year Book Publishers, Inc., 1953, p. 314.

2. Ravitch, M. M.: Hind Gut Duplication—Doubling of Colon and Genital Urinary Tracts, Ann. Surg. 137: 588 (May) 1953.

# Experimental Aberrant Lipogenesis

*I. Serum Factor*

DAVID G. COGAN, M.D., and TOICHIRO KUWABARA, M.D., Boston

By aberrant lipogenesis is meant fat synthesis by cells which do not normally form fat in sufficient aggregation to be histologically evident.* In the present paper we are concerned with that aberrant lipogenesis which may be induced by exposing a tissue to sodium oleate and serum, as described in previous preliminary reports.[1,2]

Although many tissues have the obligate property of forming fat when exposed to sodium oleate (or other oleate-containing substance), the cornea has been particularly suitable for the study of fat synthesis, inasmuch as it has a uniform cytologic architecture and yields fat formation reproducibly. It is, moreover, particularly suitable for evaluation of the serum factor on account of its normal avascularity. Most of the studies reported in this paper were accordingly made on the cornea, but observations on other tissues, to be reported separately, have shown the oleate-induced lipogenesis to be a widespread phenomenon with potential relevance to the origin of fat in fatty degeneration and in atherosclerosis.

Lipogenesis was initially induced by injecting oleate-containing substances into the corneas of intact animals. The essential role of serum in this process was not appreciated until lipogenesis was induced in excised buttons. It was then found that lipogenesis occurred only in the presence of serum. The present set of experiments, reported in this paper, were directed to finding out what factor or factors in serum were necessary for lipogenesis. This will be referred to as the serum factor, in contrast to the substrate or oleate factor discussed in a subsequent paper.

## Present Investigation

The technique consisted simply of placing excised buttons of rabbit corneas in tubes containing 3 ml. of test solution and incubating them under sterile conditions at 37 C for 24-48 hours. Ten to twenty buttons† could be obtained from each cornea. The sodium oleate was either injected into the corneal button (about 0.01 ml. of 10% solution) or added to the test solution (2 mg/ml. of serum). The test solution was varied, as will be described, for specific purposes. At the end of each experiment, the buttons were fixed in 5% neutral formalin, sectioned in the frozen state, and stained with Sudan IV or Oil Red O.

When a corneal button is thus incubated in serum-oleate medium, sudanophilic globules first become recognizable in 6 hours and are abundant in 24-48 hours. The globules are exclusively intracellular, being found first about the nuclei, and come to occupy a large part of the cytoplasm. They are found in all the cells of the cornea (epithelium, stroma, and endothelium) but are most conspicuous in the epithelium, where the cell population is the densest. This lipogenesis is the result of an enzymatic process occurring in the pH range of 6.8 to 8.0.

Submitted for publication Dec. 12, 1956.

From the Howe Laboratory of Ophthalmology, Harvard Medical School, and the Massachusetts Eye and Ear Infirmary.

This work was aided by grants from the Greater Boston Chapter of the Massachusetts Heart Association, and the American Heart Association.

*Lipogenesis is often used, especially in the biochemical literature, to refer to synthesis of fatty acids from acetate. The present use of the term refers to synthesis of neutral fats, and has no bearing on the synthesis of fatty acids.

†The use of the word buttons in this text is not strictly correct but is being used for lack of a better alternative. Initially buttons were trephined from the cornea, but subsequently it was found that greater economy in tissue was obtained by cutting up the cornea into suitable pieces. These continued to be called buttons.

Fig. 1.—Lipogenesis resulting from injection of sodium oleate into an excised cornea followed by incubation for five days in serum. Approximately 0.05 ml. of 10% sodium oleate was injected into the cornea at the extreme left. The proximal corneal cells have almost entirely disappeared, and this area is surrounded by a zone of cells which are loaded with sudanophilic material. All the fat present was within cells, some of which were grotesquely distended.

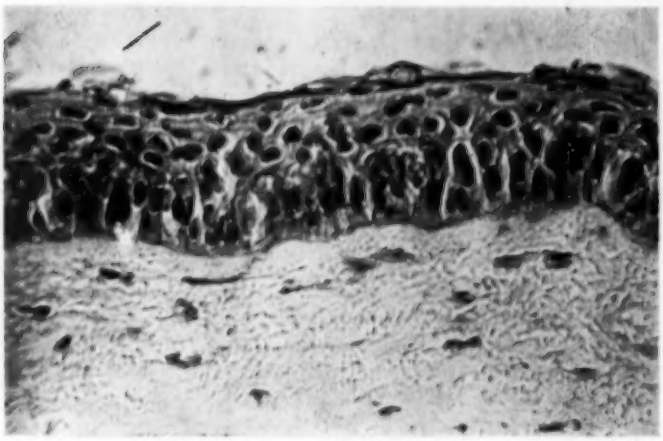


Fig. 2.—Epithelium and superficial stroma of a rabbit cornea which had been incubated in a serum-sodium oleate mixture for only three hours, then fixed in formalin, sectioned in a frozen state, and stained with hematoxylin and Sudan. No lipid was demonstrable at this stage.

In the following experiments, the oleate factor was kept as constant as possible. The variables refer to the serum or to the condition of incubation; unless stated otherwise, the oleate concentration was the same for all experiments (approximately 2 mg/ml.) and will not be referred to further. The serum was obtained, unless stated otherwise, by cardiac puncture from rabbits and separated from red blood cells by centrifuging and decanting.

*A. Species Type of Serum.*—Lipogenesis occurred in the rabbit cornea equally well when incubated with sera from rabbit, guinea pig, horse, and human sources. Similarly, lipogenesis occurred in the human or guinea pig cornea with sera from rabbit, horse, or human sources.

*B. Dilution of Serum.*—Dilution of serum with 0.9% sodium chloride resulted in a progressive weakening of the lipogenic factor. This was already evident in a twofold dilution, while complete absence of lipogenesis occurred in dilutions of 4- to sixteenfold.

To test the possibility that this weakening might be due to dilution of electrolytes, Tyrode's solution ‡ was used as a diluent instead of sodium chloride. Lipogenesis was considerably reduced by a fourfold dilution and again completely prevented by a sixteenfold dilution. Thus, the reduction in

‡ Tyrode's solution consists of physiologic proportions of sodium chloride, potassium chloride, calcium chloride, magnesium chloride, monobasic sodium phosphate, sodium bicarbonate, and dextrose.

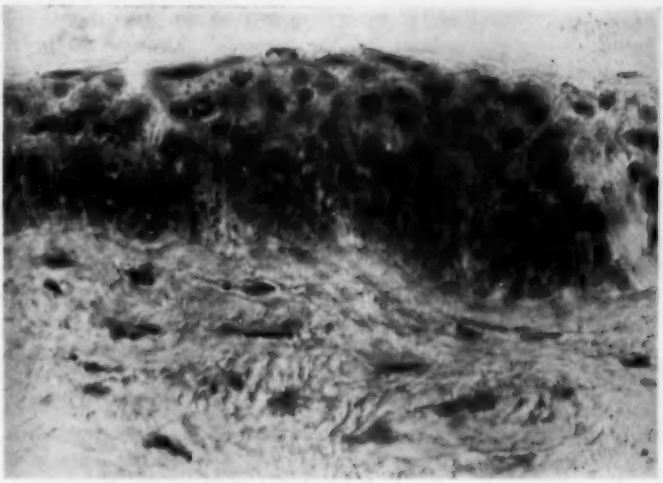


Fig. 3.—Similar preparation to that in Figure 2 which had been incubated for 24 hours. The sudanophilic globules were moderately abundant in the epithelial and stromal cells.

Fig. 4.—Similar preparation which had been incubated for three days. The epithelial and stromal cells were literally packed at this stage with sudanophilic globules.

lipogenesis was not due to reduction in electrolytes or dextrose.

Evidence accumulated elsewhere has indicated that the serum proteins "protected" the cells from the toxic effects of oleates.§ It was, therefore, thought possible that the reduction in lipogenesis with dilution might be due to the lessening of this protection. To test this possibility, serum and oleate concentrations were diluted equally. This, however, resulted in a weakening of lipogenesis similar to that which occurred with dilution of serum alone. Hence the dilution effect was not attributable to a toxic effect of the oleates.

It was inferred, therefore, that the dilution of the serum proteins was responsible for the reduction in lipogenesis.

*C. Effect of Heating, Freezing or Lyophilization.*—The serum factor withstood heating to 60 C for three hours without any deterioration in its subsequent ability to support lipogenesis. On the other hand, heating serum to 100 C greatly reduced or abolished the lipogenic factor. In this latter case most of the proteins were coagulated,

§ Thus, corneal tissue incubated in oleate-saline solution showed disintegration of the cells to a greater extent than in solutions of albumin or serum having the same oleate concentration.

again suggesting that the protein moiety of serum was one, at least, of the essential factors. What little lipogenesis did occur with incubation in the supernatant fluid appeared to depend on the residual protein (mucoprotein?), for lipogenesis did not occur when the dialysate of this supernatant fluid was used or after alcohol treatment of the supernatant fluid.

Refrigeration of serum at 5 C or freezing with dry ice-alcohol mixture (−80 C) had no effect on the subsequent ability of the serum to support lipogenesis. Nor did drying of serum in vacuo at 37 C, room temperature, or in the frozen state (lyophilization) have any constant effect on the capacity of serum to support lipogenesis.

*D. Effect of Storage or Prior Incubation of the Serum.*—Serum stored in the refrigerator at 2-4 C for two to three weeks, but not frozen, showed normal ability to support lipogenesis (even when contaminated with mold). Serum which had been incubated at 37 C for two to seven days also showed full retention of the lipogenic factor. Thus the serum factor is not appreciably destroyed by autogenous metabolic activity.

*E. Delipidization of Serum.*—Removal of most of the blood lipids by shaking with ether-alcohol or removal of the fatty acids by ether extraction at pH 2 did not affect the lipogenic factor in serum.

*F. Protein Component of Serum.*—The first approach to evaluate the protein moiety was by dialysis. Neither the dialysate alone nor the residue alone redissolved in 0.9% sodium chloride solution supported lipogenesis, but reconstitution of the residue with dialysate restored the lipogenic factor fully. It thus appeared that two factors in serum were essential, a protein component and a dialyzable component. As will be noted subsequently, the latter may be supplied by Tyrode's solution.

Further evidence for the essentiality of a protein component was the fact that lipogenesis did not occur with serum from which the protein had been removed by trichloroacetic acid, ammonium sulfate, or alcohol precipitation.

Nevertheless, full identification of the responsible protein or proteins has been unsuccessful, for no significant lipogenesis has been produced by addition of the following proteins to 0.9% sodium chloride or to Tyrode's solution in concentrations approximating that which occurs in serum: commercial crystallized albumin, obtained from The Armour Laboratories; albumin-oleic acid, obtained from Microbiologic Associates, Bethesda, Md., or prepared according to the method of Dubos and Middlebrook[3]; albumin obtained by $(NH_4)_2SO_4$ fractionation; globulin obtained by $(NH_4)_2SO_4$ fractionation or albumin-globulin obtained by $(NH_4)_2SO_4$ fractionation; Cohn's serum factors I through V individually and collectively (fibrinogen $\alpha$, $\beta$, and $\gamma$ globulin, albumin, and lipoprotein); mucoprotein extracted from beef cornea (by ammonia or calcium chloride); serum mucoprotein,‖ or serum lipoprotein.¶

The foregoing is interpreted as indicating either that the essential protein is so denatured during its preparation that it loses the lipogenic factor or else that the protein factor is also multiple.

*G. Nonprotein Component.*—The essentiality of some nonprotein component was indicated by the fact that lipogenesis occurred only when the dialysate of serum was added to the residue. Tyrode's solution could be substituted for the dialysate, but no lipogenesis occurred with substitution by solutions of sodium chloride, dextrose, or glycerin in concentrations approximating those present in serum. Moreover, no lipogenesis occurred with serum passed through a cation-exchange column, where the cations were replaced by sodium. But the lipogenic factor was largely restored by the addition of calcium chloride,# and to some extent by $MgCl_2$. Thus the essential nonprotein

‖ Prepared by Dr. Karl Schmid.[4]

¶ Prepared by Dr. Karl Schmid, using KBr and ultracentrifugation and dialysis against Tyrode's solution.

# Excess calcium, however, precipitated the oleate and diminished the subsequent lipogenesis.

components appeared to be calcium and/or magnesium.

*H. Serum Substitutes and Fortification of Serum.*—In addition to the negative results previously noted with incubation of buttons in sodium chloride, dextrose, glycerin, and Tyrode's solution, no significant lipogenesis occurred in the following solutions: phosphate buffer (pH 7.49); hemoglobin; aqueous humor; polysorbate 80 (Tween 80) in Tyrode's solution *; Simms' lipfanogen,† and macerated extracts of liver, heart, kidney, and spleen suspended in Tyrode's solution.

No definite increase in lipogenesis occurred by fortifying serum with dextrose, glycerin, calcium chloride, or magnesium chloride or by oxygenating the serum during incubation.

## Comment

To our knowledge, the synthesis of neutral fat has not been previously studied in preparations such as here described. However, fat formation has long been observed in tissue cultures,[6] where it has been generally regarded as a manifestation of degeneration or lipophagocytosis. Rarely has it been suggested that a dynamic synthesis might be involved, and, with the exception of a series of experiments by Simms et al.,[5,8] the essentiality of serum and other factors has not been systematically investigated. Simms isolated a substance from plasma called a lipfanogen which produced fat in chicken fibroblasts on incubation. We were unable to induce fat formation in our preparations when this lipfanogen was substituted for serum. It is entirely possible, however, that the lipfanogen contains the substrate factor (to be discussed in the subsequent paper) rather than what we have called the serum factor.

The exact role of serum in lipogenesis is not clear. It does not appear to be essential for the provision of dextrose, glycerin, lipids, or enzymes. A real possibility is that it provides a vehicular service for transport of the oleate across the cell wall, for oleic acid is known to combine readily with serum albumin[9] and other blood constituents[10] for transport elsewhere in the body. It is clearly different from lipogenesis with pancreatic lipase in vitro, where triolein may be synthesized from oleic acid and glycerin in the absence of serum.[11]

## Summary and Conclusions

The serum factor for experimental lipogenesis is species nonspecific.

Attenuation of the serum factor on dilution is apparent with dilutions of two- to four-fold. This dilution effect is due to reduction of the protein moiety, but not to removal of the "protective" colloids.

The serum factor withstands heating to 60 C, freezing, or lyophilization. On the other hand, heating sufficiently to coagulate most of the proteins (100 C) practically abolishes the lipogenic effect.

The serum factor is not in the lipid-extractable fraction.

The serum factor requires both a dialyzable and a nondialyzable component. The former appears to be the calcium and magnesium ions. The latter has not been convincingly identified, but it does not reside in any single protein moiety.

243 Charles St. (14).

## REFERENCES

1. Cogan, D. G., and Kuwabara, T.: Lipogenesis by Cells of the Cornea, Science 120:321-322, 1954.

2. Cogan, D. G., and Kuwabara, T.: Lipogenesis by Cells of the Cornea, A. M. A. Arch. Path. 59:453-456, 1955.

3. Dubos, R. J., and Middlebrook, G.: Media for Tubercle Bacilli, Am. Rev. Tuberc. 56:334-345, 1947.

* Polysorbate 80 had previously been found to be a substrate for lipogenesis when injected into the cornea in vivo. In the present experiments it was used to test the possibility that binding of the oleate, analogous to that which occurs with serum albumin, was essential for lipogenesis. This was found not to be the case.

† Simms' lipfanogen[5] is an unidentified substance occurring in chicken and human plasma on incubation and giving rise to fat in cells of tissue culture.

4. Schmid, K.: J. Am. Chem. Soc. 75:60, 1953.

5. Simms, H. S., and Stillman, N. P.: Production of Fat Granules and of Degeneration in Cultures of Adult Tissue by Agents from Blood Plasma, Arch. Path. 23:316, 1937.

6. Lewis, M. R., and Lewis, W. H.: Cultivation of Tissue from Chick Embryos, Anat. Rec. 5:277, 1911.

7. Lambert, R. A.: Fat Metabolism in Tissue Cultures, Proc. New York Path. Soc. 14:194, 1914.

8. Simms, H. S., and Sanders, M.: Use of Serum Ultrafiltrate in Tissue Cultures for Studying Deposition of Fat and for Propagation of Viruses, Arch. Path. 33:619, 1942.

9. Gordon, R. S.: Interaction Between Oleate and Lipoproteins of Human Serum, J. Clin. Invest. 34:477, 1955.

10. Hall, G. V.: Pathogenesis of Atheroma, with Special Reference to Disordered Lipoid Metabolism, M. J. Australia 2:475, 1956.

11. Artoon, C., and Réale, L.: Sur les produits de la synthèse entre l'acide oléique et le glycerol par la lipase du pancréase, Bull. Soc. chim. biol. 18:959, 1936.

# Cross-Circulation and Temporary Arteriovenous-Shunt Lesions in Rabbits

RICHARD L. DAVIS, M.D., JOEL G. BRUNSON, M.D., and PETER E. FEHR, B.A., Minneapolis

Previous studies have shown that diffuse vascular fibrinoid lesions may be produced in rabbits by two properly spaced intravenous injections of Gram-negative bacterial endotoxin,[1] or by a single intravenous injection of endotoxin in conjunction with the administration of such substances as cortisone,[2] colloidal thorium dioxide (Thorotrast),[3] colloidal iron,[4] or certain high molecular weight acidic polymers, such as sodium polyanethol sulfonate (Liquoid).[5,6] The mechanisms whereby such lesions are produced have been intensively investigated.[1-10]

Morphologic study of the development of the lesions suggested that fibrinoid or its precursors was formed in and deposited from the circulating blood. The demonstration that the lesions could be prevented by large amounts of heparin[6,11] strengthened this hypothesis.

In addition, the discovery of a heparin-precipitable protein which appears in the plasma of rabbits after a single intravenous injection of endotoxin[7] is of interest. Present evidence indicates that this protein is an altered form of fibrinogen. This material almost completely disappears after an intravenous injection of such high molecular weight polymers as sodium polyanethol sulfonate, and there is a concomitant fall in blood fibrinogen levels.[8] Both of these changes have been correlated with the development of diffuse fibrinoid lesions in rabbits, and these data further strengthen the hypothesis of the derivation of fibrinoid from the blood. Finally, in studies in which isolated kidneys were perfused with the blood of properly prepared donor animals, it was shown that typical fibrinoid material was deposited in the perfused organ, thus demonstrating that this material or its precursors are carried by the blood.[9]

A previous report has described the development of isolated cardiac fibrinoid lesions in rabbits subjected to carotid-jugular cross-circulation or temporary arteriovenous-shunt procedures.[12] Data obtained in those studies suggested that chemical factors operated in the production or deposition of fibrinoid, and that mechanical factors which were associated with damage to the myocardium and valves prepared these sites for the localization of fibrinoid or its precursors.

The decreased incidence of the lesions when large amounts of heparin were used with the shunting procedures suggested that another anticoagulant might further define the processes resulting in the presence of fibrinoid in the lesions, and sodium polyanethol sulfonate was used for this purpose. The results of this experiment then prompted us to investigate the effects produced by cortisone and by Gram-negative bacterial endotoxin when given in conjunction with the cross-circulation or temporary arteriovenous-shunt procedures. These results then suggested that additional information regarding the production and deposition

Submitted for publication Sept. 25, 1956.

From the Department of Pathology, University of Minnesota Medical School.

Part of this work was done during the tenure of a Lederle Laboratories Medical Student Research Fellowship (Mr. Fehr).

These studies were supported by grants from the American and Minnesota Heart Associations, the Graduate School Research Fund of the University of Minnesota, and the U. S. Public Health Service (H-2540).

of fibrinoid material might be obtained by the use of colloidal iron or colloidal thorium dioxide (Thorotrast) in conjunction with the shunt techniques. These colloidal materials were used not only because they are associated with the production of fibrinoid when given intravenously with endotoxin but also because they are easily seen microscopically and their sites of deposition may thus be ascertained.

## Materials and Methods

Two hundred twenty (220) hybrid albino rabbits of the same stock and size as those used in previous experiments were employed in the present study. Of these, 115 were used in the experiments in which sodium polyanethol sulfonate, cortisone, or endotoxin was given in association with the shunt procedures, and 90 were given colloidal iron or colloidal thorium dioxide in conjunction with these procedures. An additional 15 animals were used as controls. These were given 2 ml. of colloidal iron or colloidal thorium dioxide intravenously, followed in one hour by an additional 1 ml. and 10 mg. of heparin sodium. Details of the numbers of animals used in the various experiments are given in the text. The animals died or were killed within 96 hours after the cross circulation or temporary arteriovenous shunts were performed. Routine postmortem examinations were made, and the tissues were fixed in 10% neutral formalin. Hematoxylin and eosin were used routinely, but many additional sections were stained by the periodic acid-Schiff method, Mallory's phosphotungstic-acid hematoxylin, toluidine blue, and Perls' method for iron.

Meningococcal endotoxin, prepared by a method described previously,[15] was used in certain of the experiments. It was diluted 1:80 with sterile, pyrogen-free isotonic saline and injected into the marginal ear vein in a volume of 2 ml.

Sodium polyanethol sulfonate* was dissolved in sterile, pyrogen-free isotonic saline, passed through a Seitz filter, and injected in amounts of 8 mg. into the marginal ear vein of the animals.

Cortisone acetate† was used in certain of the experiments. It was injected intramuscularly in a 1 ml. volume (25 mg.) daily for four days, and the cross-circulation procedure was performed on the fourth day.

Except for those animals given sodium polyanethol sulfonate, heparin sodium was used as the anticoagulant in all shunting procedures. It was injected in a volume of 1 ml. (10 mg.) into the marginal ear vein of the animals just prior to cannulation of the neck vessels.

Saccharated iron oxide (Proferrin‡), containing 20 mg. of available iron per milliliter, was used in 1 or 2 ml. quantities. It was injected intravenously as above, or into the venous catheter, one hour prior and just prior to shunting. A colloidal suspension of thorium dioxide,§ containing 24% to 26% stabilized colloidal thorium dioxide by volume, was used in certain of the experiments. It was injected in a manner and volume similar to that of saccharated iron oxide.

Cross-circulation experiments were performed by connecting the carotid artery of one animal with the external jugular vein of a second animal, and the carotid artery of the second animal to the jugular vein of the first, by means of polyethylene tubing. The details of this procedure have been reported previously.[9,13] Circulation was allowed to continue for a maximum period of 45 minutes in any one pair of animals. Of the total number of rabbits, 138 were subjected to cross circulation in this fashion.

Arteriovenous shunts were carried out as previously described[12] by connecting the carotid artery and the external jugular vein of the same animal by means of polyethylene tubing. Sixty-seven animals were subjected to this procedure, and circulation through the shunt did not exceed 30 minutes in any animal.

Upon termination of the shunting procedures, the tubing was checked for patency and free flow, the cannulae were removed, and the neck vessels which had been cannulated were ligated.

## Results

The results of these experiments are summarized in Tables 1 and 2. From these Tables it may be seen that, while cardiac fibrinoid lesions occurred in almost all groups of animals, there were also extracardiac lesions. These lesions consisted of the presence of fibrinoid material in the renal glomerular capillaries, splenic sinusoids, and pulmonary arteries, and areas of focal necrosis in the liver. The incidence and severity of the lesions varied somewhat from group to group, but the changes were morphologically similar in all groups.

*Liquoid was obtained from Hoffmann-La Roche, Inc., Nutley, N. J.

†Obtained from The Upjohn Company, Kalamazoo, Mich.

‡Sharp & Dohme, Division of Merck & Co., Inc., Philadelphia.

§Thorotrast was obtained from Testagar & Company, Inc., Detroit; Lot No. 07107.

TABLE 1.—*Incidence of Lesions Produced by Cross Circulation or Temporary Arteriovenous Shunts in Conjunction with the Administration of Sodium Polyanethol Sulfonate (Liquoid), Endotoxin,* or Cortisone†*

| Procedure | Duration of Shunt,‡ Min. | Anticoagulant, Mg. | Other Material | No. of Animals | Dead in 12 Hr, % | Cardiac Lesions, % | Fibrinoid | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Heart, % | Kidney, % | Spleen, % | Lungs, % |
| Cross Circulation | 29 | Liquoid, 8 | None | 24 | 29 | 54 | 20 | 80 | 8 | 75 |
| | 30 | Liquoid, 8 | Toxin 18 hr. prior | 6 | 50 | 67 | 0 | 33 | 67 | 67 |
| | 28 | Heparin Sodium, 10 | Toxin 18 hr. prior | 14 | 14 | 50 | 29 | 0 | 43 | 0 |
| | 19 | Heparin Sodium, 10 | Toxin 18 hr. after | 10 | 90 | 90 | 80 | 10 | 80 | 80 |
| | 24 | Heparin Sodium, 10 | Cortisone | 24 | 38 | 46 | 33 | 8 | 21 | 25 |
| Arteriovenous Shunt | 26 | Liquoid, 8 | None | 8 | 38 | 75 | 38 | 38 | 0 | 100 |
| | 24 | Liquoid, 8 | Toxin 18 hr. prior | 7 | 43 | 71 | 29 | 29 | 57 | 71 |
| | 29 | Heparin Sodium, 10 | Toxin 18 hr. prior | 11 | 9 | 73 | 18 | 0 | 18 | 9 |
| | 30 | Heparin Sodium, 10 | Toxin 18 hr. after | 11 | 27 | 91 | 55 | 9 | 64 | 9 |

* Two milliliters of 1:80 dilution.
† Twenty-five milligrams intramuscularly for four days, with procedure performed on fourth day.
‡ Average duration of shunt for group.

The cardiac lesions were similar to those described in the previous groups of animals subjected only to cross-circulation or temporary arteriovenous-shunt procedures.[12] They consisted of myocardial hemorrhage, muscle necrosis and calcification, mononuclear and heterophilic cellular reaction, and fibrinoid deposition in the coronary arterial walls, lumens, and perivascular spaces. Valvular hemorrhage, edema, and cellular reaction were observed also, together with large deposits of fibrinoid material within the substance or along the free margins of the valves (Figs. 1, 2, 3).

One major difference was noted, however, in the cardiac lesions in the animals in which the shunt procedures had been performed in conjunction with the administration of saccharated iron oxide or colloidal thorium dioxide. This consisted of the presence of iron or thorium dioxide lying in the areas of myocardial hemorrhage and necrosis and admixed with the fibrinoid material in the arteries, valves, and perivascular spaces (Figs. 6-13).

In sections stained with hematoxylin and eosin, the iron appeared as a brownish, slightly granular material. In alternate sections stained by the periodic acid-Schiff method and by Perls' method for iron, it was observed that the iron and the Schiff-positive fibrinoid material were intimately associated and appeared to be deposited in identical segments of arteries or valves (Figs. 6, 7, 10, 11). In some sections endothelial proliferation was noted, and granules of iron appeared to be within the cytoplasm of these cells.

Similarly, in the rabbits given colloidal thorium dioxide brownish, refractile globules of this material could be seen mixed with the fibrinoid material beneath the arterial endothelium and within the valves (Figs. 9, 12, 13). Granules of this material also appeared to lie within the cytoplasm of proliferating endothelial cells. The cellular reaction to the thorium dioxide appeared to be more extensive than in the animals given saccharated iron oxide, and large numbers of heterophils and mononuclears were observed about the thorium dioxide-fibrinoid lesions.

Gross renal cortical necrosis, resembling that seen in rabbits given endotoxin in association with sodium polyanethol sulfonate,[5,6] occurred only in the group of rabbits in which the shunting procedures were performed using sodium polyanethol sulfonate as the anticoagulant. In other groups fibrinoid was present in scattered glomeruli but usually did not involve all of the capillary loops of any one glomerulus (Fig. 4). The renal lesion was somewhat more diffuse in the animals given saccha-

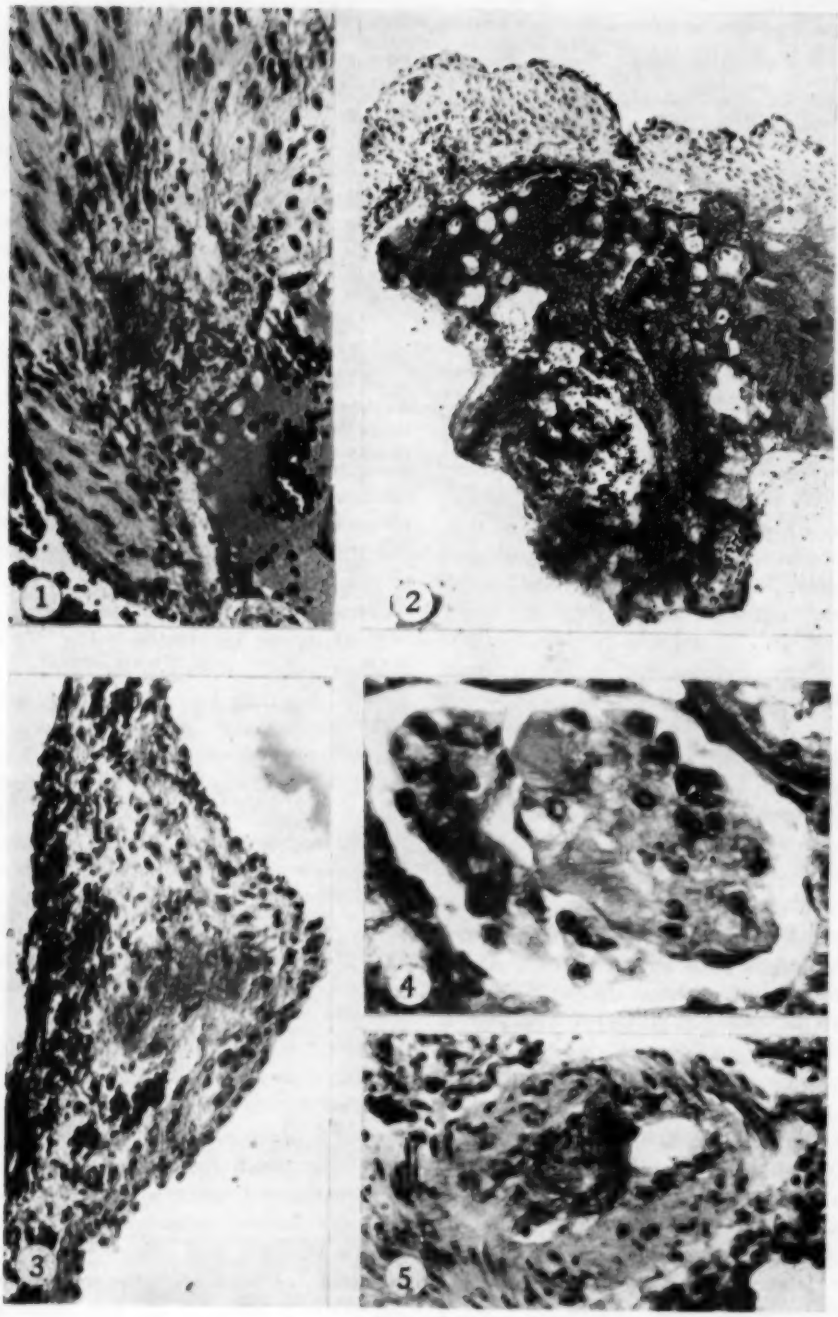



PLATE I

TABLE 2.—*Incidence of Lesions Produced by Cross Circulation or Temporary Arteriovenous Shunts in Conjunction with Administration of Saccharated Iron Oxide (Proferrin)* or Colloidal Thorium Dioxide (Thorotrast)**

| Procedure | Duration of Shunt,† Min. | Other‡ | No. of Animals | Dead in 12 Hr., % | Cardiac Lesions, % | Fibrinoid | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Heart, % | Kidney, % | Spleen, % | Lungs, % |
| Cross circulation | 29 | Proferrin 2 ml.+1 ml. | 34 | 23 | 79 | 68 | 55 | 41 | 41 |
| | 19 | Proferrin 2 ml.+0 ml. | 8 | 25 | 88 | 50 | 25 | 0 | 25 |
| | 28 | Thorotrast 2 ml.+1 ml. | 18 | 11 | 89 | 72 | 61 | 72 | 33 |
| Arteriovenous shunt | 26 | Proferrin 2 ml.+1 ml. | 8 | 13 | 88 | 50 | 13 | 25 | 25 |
| | 28 | Proferrin 0 ml.+2 ml. | 6 | 17 | 100 | 50 | 17 | 0 | 33 |
| | 30 | Thorotrast 2 ml.+1 ml. | 16 | 13 | 69 | 38 | 56 | 88 | 19 |
| None | None performed | Proferrin 2 ml.+1 ml. | 7 | 0 | 0 | 0 | 0 | 71 | 0 |
| | | Thorotrast 2 ml.+1 ml. | 8 | 0 | 13 | 0 | 13 | 100 | 0 |

* The first volume was injected intravenously one hour prior to the second; if a procedure was performed, it followed the second injection immediately.
† Average duration of shunt for the group.
‡ All animals in these groups were given 10 mg. of heparin sodium intravenously at the time of the second injection of saccharated iron oxide or colloidal thorium dioxide.

rated iron oxide or colloidal thorium dioxide, but tubular necrosis was observed in only one animal. The fibrinoid material was mixed, as in the heart, with iron or thorium dioxide (Fig. 14), and such mixtures were observed occasionally in the lumens and walls of the interlobular arteries (Fig. 15).

The spleens of the animals given either saccharated iron oxide or colloidal thorium dioxide in conjunction with the shunt procedures were grossly enlarged and firm on palpation, but no areas of infarction were observed. In those given colloidal thorium dioxide the organ appeared larger than in those given saccharated iron oxide, but this change was not quantitated. Microscopically, large amounts of the injected material, together with fibrinoid, were observed in the splenic sinusoids. Granules of iron or thorium dioxide were present also within the sinusoidal lining cells, and occasionally the cells contained a hyaline material, which appeared to be fibrinoid. Similar splenic changes were observed in the control animals given 3 ml. of either saccharated iron oxide or colloidal thorium dioxide (Table 2). In the spleens from the other groups of animals no gross changes were evident, but fibrinoid was observed microscopically as localized deposits lying in the sinusoids.

Fibrinoid material was observed also in branches of the pulmonary arteries, often associated with marked endothelial proliferation (Fig. 5). In many animals this material was observed to lie beneath the endothelium of the vessel, mixed with iron or thorium dioxide (Figs. 16, 17).

Microscopic examination of the liver showed numerous areas of focal necrosis, with and without cellular reaction. These

Fig. 1.—Section from the mitral valve of an animal subjected to cross circulation and given an intravenous injection of endotoxin 18 hours later. There is a palisading of inflammatory cells about a mass of fibrinoid material within the substance of the valve. Hematoxylin-eosin stain; × 250.

Fig. 2.—Section from the aortic valve of an animal given an intravenous injection of endotoxin 18 hours prior to cross circulation, sodium polyanethol sulfonate being used as the anticoagulant. A large thrombus of fibrinoid material is attached to the free margin of the valve leaflet. Hematoxylin-eosin stain; × 100.

Fig. 3.—Section from the mitral valve of a rabbit given cortisone prior to the cross-circulation procedure. Extensive hemorrhage and fibrinoid deposition are present, but there is a relative absence of edema and cellular reaction. Hematoxylin-eosin stain; × 250.

Fig. 4.—Renal glomerulus from an animal given cortisone prior to cross circulation. A large number of the glomerular capillaries appear occluded by fibrinoid material. Hematoxylin-eosin stain; × 450.

Fig. 5.—Pulmonary artery from a rabbit given an intravenous injection of endotoxin 18 hours after arteriovenous shunt. A mass of fibrinoid material occludes part of the vessel lumen. Most of this mass appears to be covered by proliferating endothelial cells. Hematoxylin-eosin stain; × 400.

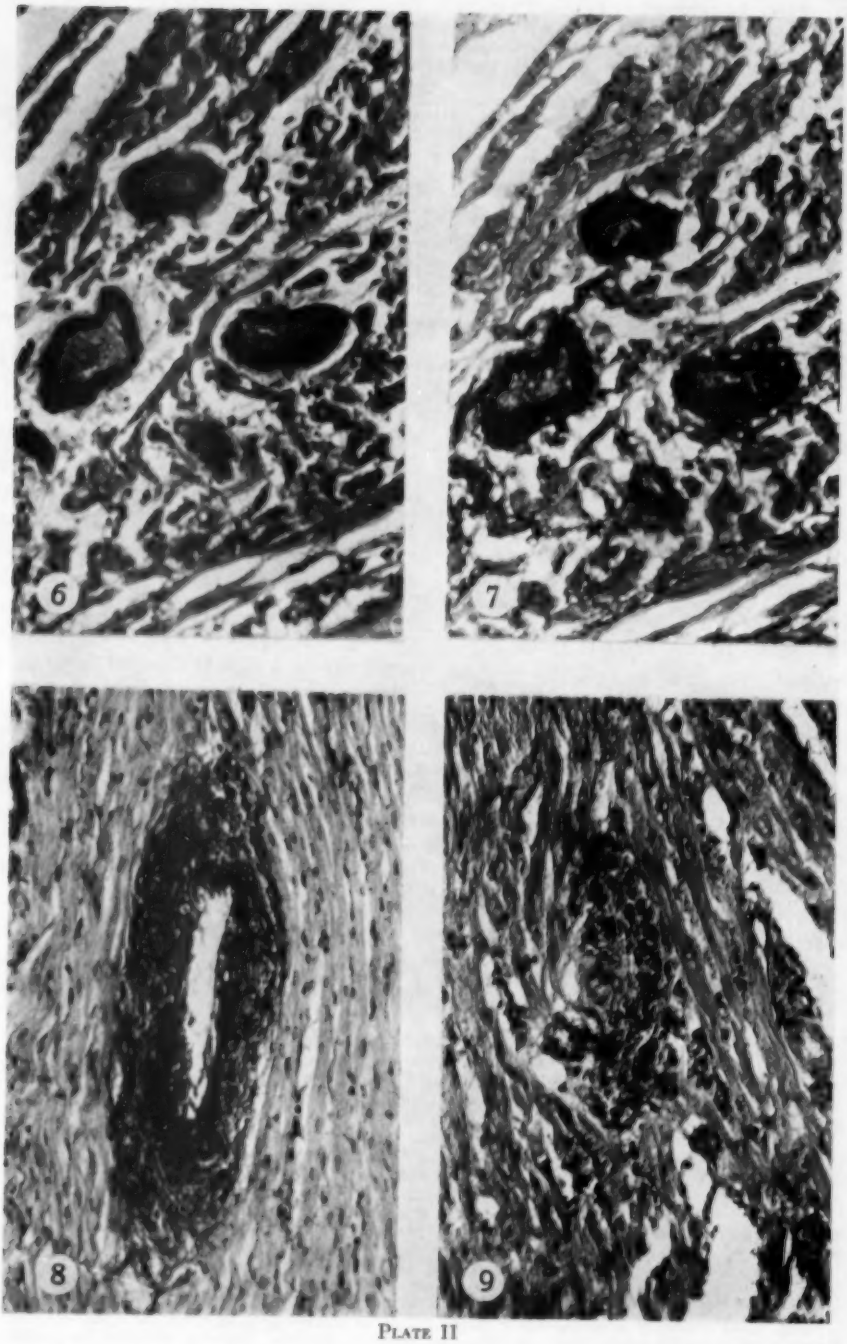



PLATE II

areas were not localized to any particular part of the lobule, and they appeared to be ischemic in nature, although thrombi were seldom seen. In most animals, however, only a single section of liver was examined, and more thrombi might have been detected had multiple sections been studied. In the animals given colloidal thorium dioxide, there was an active cellular reaction, and in many cases granulomas composed of mononuclear and giant cells were present about the clumps of thorium dioxide. In the group of animals in which iron or thorium dioxide was give in conjunction with the shunting, fibrinoid material was observed occasionally within the hepatic sinusoids and in branches of the hepatic artery, and, as in other organs, this material was mixed with the injected compound.

In an occasional animal, microscopic sections of the brain showed the presence of clumps of fibrinoid, admixed with iron or thorium dioxide, within the capillaries of the choroid plexus. This lesion was similar to that described in animals given endotoxin in conjunction with the administration of sodium polyanethol sulfonate.[5]

The ears of the animals in the saccharated iron oxide-colloidal thorium dioxide group of experiments were noted to become blue and edematous within a period of 18 hours after the shunting procedure. Microscopically, extensive edema and hemorrhage were observed, and there were massive accumulations of fibrinoid and iron or fibrinoid and thorium dioxide within the vessels and perivascular spaces. These changes were more extensive in the ear in which the injections had been given, and possibly were related to the trauma associated with this procedure.

In two animals given saccharated iron oxide prior to shunting, psoas muscle hemorrhage was seen at autopsy. Microscopic examination showed extensive hemorrhage, necrosis, and deposits of fibrinoid and iron within the vessels and interstices, associated with the presence of numerous heterophilic cells.

## Comment

The production of isolated cardiac fibrinoid lesions in rabbits subjected to carotid-jugular cross circulation or temporary arteriovenous shunts has been described in a previous paper.[12] The results of these experiments suggested that another anticoagulant might give further information regarding the pathogenesis of the lesions. Sodium polyanethol sulfonate was used because it is a potent anticoagulant and, further, it had been demonstrated that the injection of this material in conjunction with endotoxin led to the production of diffuse fibrinoid lesions in rabbits.[5,6] This, indeed, proved to be the case in the present experiments, as a high percentage of animals in which this material was used in conjunction with the shunting procedures developed fibrinoid lesions of the kidneys and lungs, which eclipsed the cardiac fibrinoid lesions. These results suggested that the cross-circulation or arteriovenous-shunt procedures might be acting in a manner similar to that of an intravenous injection of Gram-negative endotoxin.

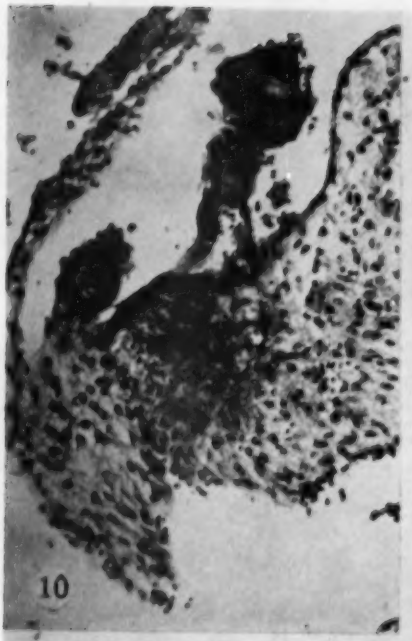
To explore this possibility, a series of experiments were performed in which endo-

Fig. 6.—Section from the myocardium of a rabbit given colloidal iron prior to the shunting procedure. Stained by Perls' method for iron. There is extensive deposition of dark-staining iron within the walls of the intramural coronary arteries. × 200.

Fig. 7.—Alternate section of myocardium from same animal as that in Figure 6, stained by the periodic acid-Schiff method. The Schiff-positive fibrinoid material almost completely replaces the walls of the vessels in the same location as the iron in Figure 1. × 200.

Fig. 8.—Intramural coronary artery from a rabbit given colloidal iron and subjected to cross circulation. Stained by Perls' method for iron. There is massive segmental deposition of iron beneath the arterial endothelium. × 200.

Fig. 9.—Section from the myocardium of an animal given colloidal thorium dioxide prior to the shunting procedure. An extensive accumulation of thorium dioxide is present within an area of focal necrosis. Refractile granules of the material are present within the cytoplasm of mononuclear and heterophilic cells. Hematoxylin-eosin stain; × 200.



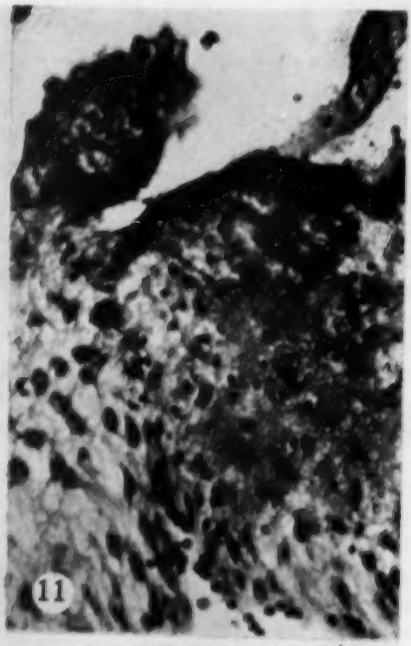



PLATE III

TABLE 3.—*Comparison of Incidence of Fibrinoid Lesions Produced by Cross Circulation or Arteriovenous Shunts Alone with That Produced by the Shunting Procedures in Conjunction with Administration of Liquoid, Endotoxin, Cortisone, Colloidal Iron, or Thorotrast**

| Procedure | Anticoagulant, Mg. | Other | No. of Animals | Fibrinoid Lesions | | | |
|---|---|---|---|---|---|---|---|
| | | | | Heart, % | Kidney, % | Spleen, % | Lungs, % |
| Cross circulation | Heparin Sodium, 10† | ..... | 22 | 45 | 0 | 0 | 0 |
| | Heparin, 40† | ..... | 16 | 6 | 0 | 0 | 6 |
| | Liquoid, 8* | ..... | 24 | 20 | 80 | 8 | 75 |
| | Heparin, 10 | Endotoxin prior | 14 | 29 | 0 | 43 | 0 |
| | Liquoid, 8 | Endotoxin prior | 6 | 0 | 33 | 67 | 67 |
| | Heparin, 10 | Endotoxin after | 10 | 30 | 10 | 80 | 30 |
| | Heparin, 10 | Cortisone | 24 | 33 | 8 | 21 | 25 |
| | Heparin, 10 | Proferrin | 34 | 68 | 56 | 41 | 41 |
| | Heparin, 10 | Thorotrast | 18 | 72 | 61 | 72 | 33 |
| Arteriovenous shunt | Heparin, 10† | ..... | 11 | 27 | 0 | 0 | 0 |
| | Liquoid, 8 | ..... | 8 | 38 | 38 | 0 | 100 |
| | Heparin, 10 | Toxin prior | 11 | 18 | 0 | 18 | 9 |
| | Liquoid, 8 | Toxin prior | 7 | 29 | 29 | 57 | 71 |
| | Heparin, 10 | Toxin after | 11 | 55 | 9 | 64 | 9 |
| | Heparin, 10 | Proferrin | 8 | 50 | 13 | 25 | 25 |
| | Heparin, 10 | Thorotrast | 16 | 38 | 56 | 88 | 19 |

* Liquoid is sodium polyanethol sulfonate; Proferrin, saccharated iron oxide, and Thorotrast, colloidal thorium dioxide.
† Previously reported data.[1,2]

toxin was given prior to and after cross-circulation or arteriovenous-shunt procedures, with in addition, the use of sodium polyanethol sulfonate or heparin sodium as the anticoagulant. The results supported this hypothesis, as a significant number of animals in each group developed renal, splenic, and pulmonary fibrinoid lesions.

Table 3 compares the incidence of fibrinoid lesions in rabbits subjected only to cross circulation or temporary arteriovenous shunts with that observed in rabbits in which these procedures were performed in conjunction with the administration of sodium polyanethol sulfonate, meningococcal endotoxin, cortisone, saccharated iron oxide, or colloidal thorium dioxide. Reference to this Table shows that there was a marked increase in the incidence of extracardiac fibinoid lesions in the animals in which any of these materials were added to the shunting procedures. Moreover, in the animals given saccharated iron oxide or thorium dioxide and subjected to cross circulation, the incidence of such diffuse lesions closely approximates that observed after two appropriately spaced intravenous injections of endotoxin, or of endotoxin in combination with sodium polyanethol sulfonate, saccharated iron oxide, or colloidal thorium dioxide.[3-6] In other experiments it was noted that when endotoxin was administered intravenously to rabbits given saccharated iron oxide or colloidal thorium dioxide, these materials were localized in the walls of vessels, as well as in conventional sites, such as the liver and spleen.[1] A similar localization was observed in the present experiments.

It thus appears that cross circulation or arteriovenous shunts may act much like either a "preparing" or a "shocking" injec-

Fig. 10.—Mitral valve from a rabbit given saccharated iron oxide prior to shunting. Combined periodic acid-Schiff and Perls' iron stain, showing the localization of the dark-staining iron with the Schiff-positive fibrinoid material beneath the endothelium of the valve. Deeper in the substance of the valve another accumulation of iron-free fibrinoid material may be seen. The valve also shows edema and increased cellularity. A mass of fibrinoid projects from the free margin of the valve. × 100.

Fig. 11.—Higher magnification of portion of valve in Figure 10, showing close association of iron and fibrinoid within the valve. × 300.

Fig. 12.—Mitral valve from a rabbit given colloidal thorium dioxide prior to shunting. Diffuse hemorrhage and cellular reaction are present, together with extensive deposits of thorium dioxide-fibrinoid within the substance of the valve. Hematoxylin-eosin stain; × 80.

Fig. 13.—Higher magnification of portion of valve in Figure 12, showing accumulation of refractile granules of thorium dioxide admixed with fibrinoid material. The more darkly staining erythrocytes can also be seen. × 150.

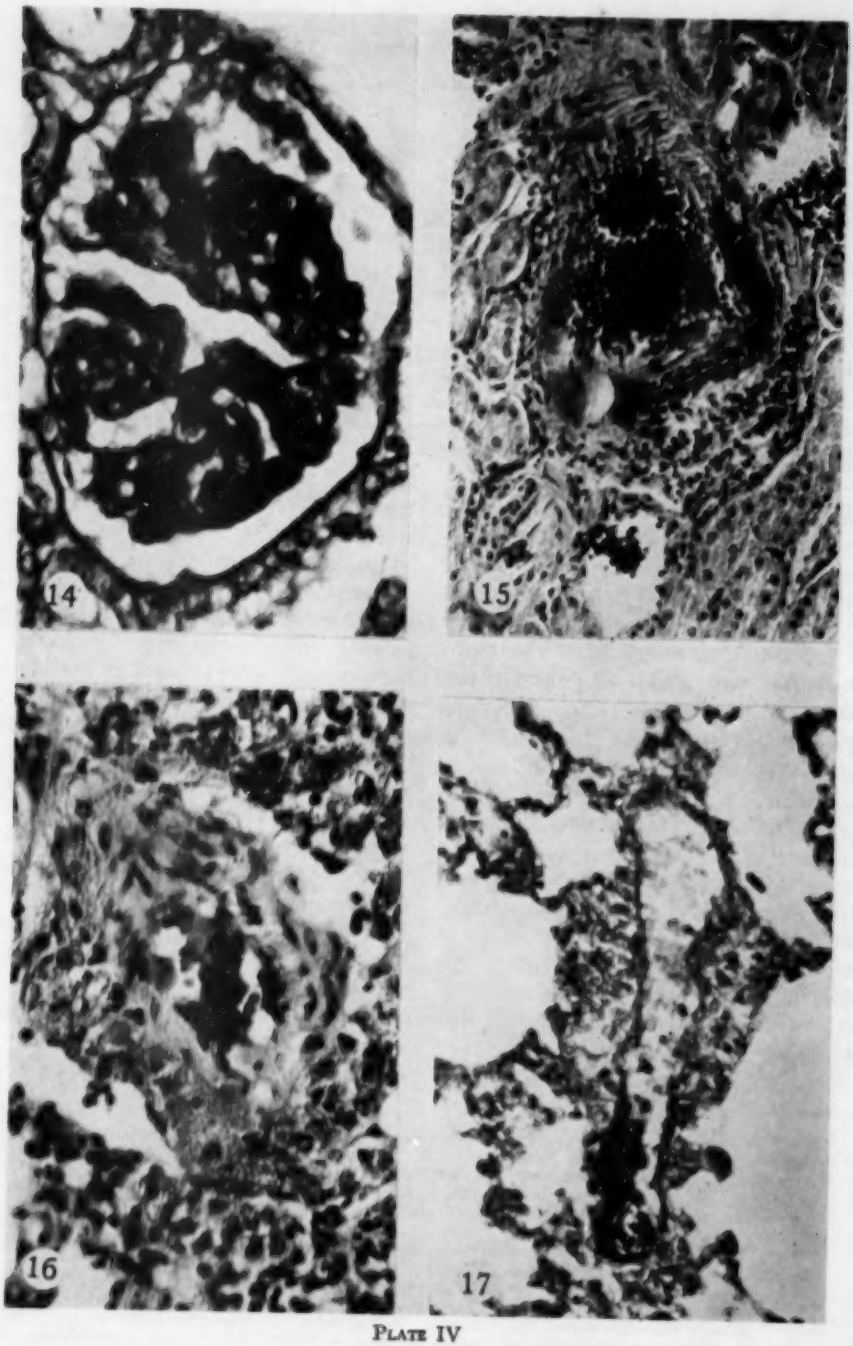



PLATE IV

tion of endotoxin, inasmuch as diffuse fibrinoid lesions were produced by the shunting procedures when they were performed in conjunction with the administration of substances which, when given in combination with endotoxin, regularly produce such diffuse lesions.

It has been suggested that fibrinoid or its precursors are deposited in sites of endothelial alteration or vascular damage.[12,14] It would appear, therefore, that these shunt procedures not only provide a method of producing localized cardiac injury but also initiate the mechanisms whereby fibrinoid or its precursors are produced and deposited in the damaged sites. These results also suggest that the amount of fibrinoid, as judged from the extent and severity of the lesions, may be a factor in determining the sites of its deposition. The production of large amounts of this material, such as occurs when sodium polyanethol sulfonate is given in conjunction with endotoxin, results in extensive deposits in the spleen and kidneys.[5] A similar result occurs when two injections of endotoxin are given at an optimal interval of time.[1] In the present experiments, no fibrinoid was observed in the spleens of the animals given only 2 ml. of saccharated iron oxide or colloidal thorium dioxide, but extensive deposits were commonly seen in the spleens of animals given 3 ml. of either of these materials. These observations suggest that, in addition to an active deposition of fibrinoid in sites of vascular damage, there may also be a passive deposition in the presence of large amounts of this material, which allows it to collect within sinusoidal and vascular lumens. Which of these mechanisms operates with respect to the lesions described here is not known with certainty, but the results indicate that an active deposition occurs in the heart. Previously reported evidence suggests that a passive mechanism may operate in the development of the renal lesion.[9]

In the present experiments, either iron or colloidal thorium dioxide was injected directly into the blood stream, and granules of the injected compound were observed within the phagocytic cells of the spleen and liver, mixed with the fibrinoid in their sinusoids, and localized in sites of fibrinoid deposition in the coronary arterial and valvular lesions. The localization of these materials with the fibrinoid, therefore, supports the previous morphologic and biochemical evidence of the derivation of fibrinoid from the circulating blood.

The observation that granules of iron or thorium dioxide appeared to be within vascular endothelial cells is also of interest, and suggests that these cells are, or may become, actively phagocytic. Duff et al.[15] noted the presence of thorium dioxide in the endothelial cells of the aorta in rabbits fed cholesterol and given colloidal thorium dioxide intravenously, but not in control animals given only the colloidal material. Further evidence of the possible phagocytic properties of these cells is the observation that bacterial endocarditis may be produced in rabbits given streptococci and subjected to cross-circulation or temporary arteriovenous-shunt procedures.[16]

The heparin-precipitable protein [7] was not demonstrated in the plasma of animals sub-

Fig. 14.—Renal glomerulus from an animal given saccharated iron oxide prior to shunting. Combined periodic acid-Schiff and Perls' iron stain. Almost all capillaries contain deposits of dark-staining iron-fibrinoid material. × 450.

Fig. 15.—Renal interlobular artery from a rabbit given colloidal thorium dioxide prior to shunting. A mass of homogeneous thorium dioxide-fibrinoid is present within the wall of the vessel at the lower left, and the right one-half of the vessel shows extensive intramural hemorrhage. Hematoxylin-eosin stain; × 200.

16.—Pulmonary artery from an animal given colloidal thorium dioxide prior to shunting. Granules of thorium dioxide are present within the cytoplasm of surrounding cells, and also within a segment of the arterial wall. Hematoxylin-eosin stain; × 400.

Fig. 17.—Pulmonary artery from a rabbit given saccharated iron oxide prior to shunting. Perls' stain for iron. Note localization of iron beneath the endothelium of a segment of the vessel. × 400.

jected to cross circulation or temporary arteriovenous shunt when these procedures were performed in combination with the administration of cortisone or sodium polyanethol sulfonate. This, however, is not surprising, as it has previously been reported that this fraction cannot be demonstrated in animals given heparin, and it rapidly disappears when sodium polyanethol sulfonate is given intravenously. Preliminary studies, however, have shown the presence of an abnormal protein fraction, somewhat similar to the heparin-precipitable fraction, in the plasma of rabbits given saccharated iron oxide or colloidal thorium dioxide in conjunction with these shunting procedures.

With either compound a flocculant precipitate was noted on cooling the plasma to 4 C for two hours. The precipitate from the animals given saccharated iron oxide was deep amber-brown, and brown globules could be seen mixed with the brownish homogeneous material on microscopic examination. When colloidal thorium dioxide was given, the precipitate was cream-colored. These precipitated fractions did not dissolve upon warming the plasma to room temperature.

If the plasma was allowed to stand at room temperature for 8 to 10 hours, a similar precipitate formed and the plasma clotted during this time, even though it contained sufficient heparin to prevent coagulation of normal plasma. The clot so formed was rubbery in consistency and retracted poorly. Preliminary electrophoretic study of this material indicates that it migrates with the globulin fraction. Other studies to characterize this protein fraction are now in progress.

In each of the cross-circulation or temporary arteriovenous-shunt experiments, heparin or a heparin-like material, sodium polyanethol sulfonate, has been used to prevent clotting in the catheters. Thus, the active participation of heparin in the formation of fibrinoid by these shunting procedures has not been excluded. It has been shown that large amounts of heparin decrease the incidence of fibrinoid lesions,[6,11] but small amounts of such a substance may be necessary for fibrinoid production or deposition in these experimental procedures.

Although the exact mechanisms involved in the production and deposition of fibrinoid or its precursors are as yet unknown, the observations now extant indicate its formation in and deposition from the circulating blood. The results of the present experiments are further evidence in favor of this concept.

## Summary

Hybrid albino rabbits were subjected to carotid-jugular cross-circulation or temporary arteriovenous-shunt procedures, in conjunction with the administration of sodium polyanethol sulfonate (Liquoid), cortisone, Gram-negative endotoxin, colloidal iron, or colloidal thorium dioxide (Thorotrast). With each of these substances, widespread fibrinoid lesions were produced, but the incidence and severity of the lesions varied in the different groups. When sodium polyanethol sulfonate was used as anticoagulant in the shunting procedures, bilateral renal cortical necrosis resembling that seen in the generalized Shwartzman phenomenon occurred. Iron or thorium dioxide was observed to be intimately associated with the fibrinoid material in all sites when these materials were given in combination with the shunts.

The results suggest that the action of the shunting procedures is similar to that of intravenously administered endotoxin, since similar diffuse fibrinoid lesions may be produced by the administration of cortisone, sodium polyanethol sulfonate, saccharated iron oxide, or colloidal thorium dioxide in conjunction with the administration of endotoxin. It is suggested that the shunt techniques provide a method of localizing fibrinoid and colloidal materials, or microorganisms, in certain sites, especially the heart. Indirect evidence indicates that the

vascular endothelial cells may play a role in such localization.

Other factors which may operate in the localization and deposition of fibrinoid are discussed. Data obtained from these studies support the hypothesis that fibrinoid or its precursors are formed in and deposited from the circulating blood. Preliminary studies indicate that altered plasma proteins may be involved in its formation.

The photographs were made by Mr. Henry Morris.

Department of Pathology, University of Minnesota Medical School (14).

## REFERENCES

1. Brunson, J. G.; Thomas, L., and Gamble, C. N.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal Toxin: II. Two Appropriately Spaced Injections; the Role of Fibrinoid in the Generalized Shwartzman Reaction, Am. J. Path. 31:655, 1955.

2. Thomas, L., and Good, R. A.: The Effect of Cortisone on the Shwartzman Reaction, J. Exper. Med. 95:409, 1952.

3. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction: II. The Production of Bilateral Cortical Necrosis of the Kidneys by a Single Injection of Bacterial Toxin in Rabbits Previously Treated with Thorotrast or Trypan Blue, J. Exper. Med. 96:625, 1952.

4. Smith, R. T.; Thomas, L., and Good, R. A.: Generalized Shwartzman Reaction: V. Intravenous Injection of Colloidal Iron or Carbon on Response of Rabbits to Meningococcal Toxin, Proc. Soc. Exper. Biol. & Med. 82:712, 1953.

5. Brunson, J. G.; Davis, R. L., and Thomas, L.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal Toxin: III. The Effects Produced by Endotoxin in Association with Certain High Molecular Weight Acidic Polymers, Am. J. Path. 31:669, 1955.

6. Thomas, L.; Brunson, J., and Smith, R. T.: Studies on the Generalized Shwartzman Reaction: VI. Production of the Reaction by the Synergistic Action of Endotoxin with 3 Synthetic Acidic Polymers (Sodium Polyanethol Sulfonate, Dextran Sulfate, and Sodium Polyvinyl Alcohol Sulfonate), J. Exper. Med. 102, 249, 1955.

7. Thomas, L.; Smith, R. T., and Von Korff, R.: Cold-Precipitation by Heparin of a Protein in Rabbit and Human Plasma, Proc. Soc. Exper. Biol. & Med. 86, 813, 1954.

8. Thomas, L.; Smith, R. T., and Von Korff, R.: Studies on the Generalized Shwartzman Reaction: VII. The Role of Fibrinogen in the Deposition of Fibrinoid After Combined Injections of Endotoxin and Synthetic Acidic Polymer, J. Exper. Med. 102:263, 1955.

9. Gamble, C. N., and Brunson, J. G.: Experimental Fibrinoid Lesions in Rabbits: Production by Cross Transfusion and Isolated Renal Perfusion, A. M. A. Arch. Path. 60:582, 1955.

10. Beeson, P.: Effect of Reticulo-Endothelial Blockade on Immunity to the Shwartzman Phenomenon, Proc. Soc. Exper. Biol. & Med. 64:146, 1947.

11. Good, R. A., and Thomas, L.: Studies on the Generalized Shwartzman Reaction: IV. Prevention of the Local and Generalized Shwartzman Reactions with Heparin, J. Exper. Med. 97:871, 1953.

12. Davis, R. L.; Brunson, J. G., and Fehr, P. E.: Cardiac Fibrinoid Lesions Produced by Cross Circulation or Temporary A-V Shunts, Circulation Res., to be published.

13. Thomas, L., and Good, R. A.: Studies on the Generalized Shwartzman Reaction: I. General Observations Concerning the Phenomenon, J. Exper. Med. 96:605, 1952.

14. Brunson, J. G.; Gamble, C. N., and Thomas, L.: Morphologic Changes in Rabbits Following the Intravenous Administration of Meningococcal toxin: I. The Effects Produced in Young and in Mature Animals by a Single Injection, Am. J. Path. 31:489, 1955.

15. Duff, G. L.; McMillan, G. C., and Lautsch, E. V.: The Uptake of Colloidal Thorium Dioxide by the Arterial Lesions of Cholesterol Atherosclerosis in the Rabbit, Am. J. Path. 30:941, 1954.

16. Brunson, J. G.; Fehr, P. E., and Davis, R. L.: Am. J. Path. to be published.

# Acanthosis Nigricans and Carcinoma of the Stomach

BELA HALPERT, M.D., and RAYMOND G. GOTTSCHALK, M.D., Houston, Texas

Two forms of acanthosis nigricans are distinguished. One is called "malignant" because of its association with cancer, and the other is called "benign" because it lacks this association. The benign form develops in young persons, often at puberty, occurs in families, and may involve only one-half of the body. When acanthosis nigricans is associated with cancer, it usually has a symmetrical distribution. In either form the acanthosis involves areas of skin folds. These assume a velvety appearance, with the furrows exaggerated and discolored brown or black. Microscopically, the epidermal ridges are elongated, and there are abundant deposits of melanin granules in the basal layer and marked hyperkeratosis of the epidermis.

According to a survey by Curth, 420 cases of acanthosis nigricans had been reported up to 1948.[1] Of these, 208 were associated with cancer. The neoplasm occurring with the acanthosis is almost always a rapidly growing and metastasizing columnar-cell carcinoma. In 93% of the reported cases the carcinomas originated in abdominal viscera. In none of the cases surveyed by Curth did the acanthosis ever become cancerous. While cancer has been observed in about one-half of the instances of acanthosis nigricans, the reason for this association is still unknown.

In the present paper acanthosis nigricans is reported in a 55-year-old Negro man who died of carcinoma of the stomach and in whom a cyst in the sella turcica was discovered at necropsy.

Submitted for publication Oct. 31, 1956.

From the Department of Pathology, Baylor University College of Medicine, and the Veterans Administration Hospital.

Now at Veterans Administration Center, Martinsburg, W. Va., and Department of Pathology, George Washington University School of Medicine, Washington, D. C. (Dr. Gottschalk).

## Report of a Case

A 55-year-old Negro lumber-mill worker was admitted to the Veterans Administration Hospital, Houston, Texas, on Nov. 1, 1950, complaining of bloating, nausea, vomiting of coffee-ground material, right upper quadrant pain, and urinary frequency of six weeks' duration. About the same time he also noticed warty projections on the face and hands, icteric sclerae, dark urine, and liquid white stools. In the last two months his weight had decreased from 168 to 138 lb. (76.2 to 62.6 kg.). His skin had become darker over the last five months.

The patient stated that in 1944 he had postprandial epigastric pain and x-ray studies by a physician disclosed an ulcer. The pain was only partly relieved by diet and medication, and there were episodes of vomiting. His appetite was poor. In 1949 he vomited small amounts of red blood. Since that time he had had a distaste for fatty foods. Recently, the epigastric pain had become more frequent, severe, and unrelated to meals. There was no known history of cancer in the family.

At the time of admission the patient appeared chronically ill and fairly well nourished. The skin was dry, with darker areas where it was thick and deeply "crisscrossed," especially on the dor-

Fig. 1.—Acanthosis nigricans of the skin of the hands.

Fig. 2.—Acanthosis nigricans of the feet.

sum of the hands and feet. The fingers were huge, with the skin dark and thickened (Fig. 1). The hands had been square and the feet block-shaped for as long as the patient could remember (Fig. 2). The lips were greatly thickened. The thick wrinkled skin gave the face a leonine appearance. Nothing about the jaws or elsewhere suggested acromegaly. The sclerae were icteric. The mucous membrane of the mouth had a gray pigmentation. Examination of the ears, nose, throat, neck, lungs, and heart was essentially negative. The blood pressure was 118 mm. of mercury systolic and 70 diastolic; the pulse rate was 68 a minute, and the temperature 99.2 F. There was marked muscular spasm in the epigastric area. The liver was tender, smooth, and extended 6 cm. below the right costal margin. A mass was felt in the left upper quadrant. The spleen was not palpable. Rectal examination revealed external hemorrhoids and a questionable Blumer's shelf. Reflexes were physiologic.

The erythrocyte count was 3,360,000; the hemoglobin content, 11.1 gm. per 100 cc. The white blood cell count was 7200, with neutrophils 68%, lymphocytes 27%, eosinophils 3%, and monocytes 2%. The urine contained 1+ albumin and no sugar; 5 erythrocytes and 15 white blood cells per high-power field; no casts, and 0.6 to 3 Ehrlich units of urobilinogen per 100 cc. The serum bilirubin was 7.8 mg. per 100 cc.; the thymol turbidity test was 3 units; the alkaline phosphatase, 41 Bodansky units, per 100 cc. The sulfobromophthalein (Bromsulphalein) retention was 32% in 30 minutes. The total protein was 6.8 gm., with albumin 4.3 gm. and globulin 2.5 gm. per 100 cc. of serum. Prothrombin time was 55% of normal. A glucose tolerance test was within normal limits. The Kahn test was negative. There were 144 mEq. of sodium, 4.8 mEq. of potassium, 92 mEq. of chlorides, and 31 mEq. of $CO_2$ per liter of serum. Urea nitrogen was 13 mg. per 100 cc. After injection of 0.3 cc. of epinephrine solution, the eosinophil count fell from 175 to 125 per cubic millimeter. Gastric analysis revealed free HCl and blood in the fasting specimen.

Roentgenographic examination of the gastrointestinal tract disclosed a filling defect without ulceration in the antral portion of the stomach and a deformity of the duodenal bulb with 50% retention of the barium in the stomach.

The patient was considered to have obstructive jaundice due to carcinoma, possibly of the head of the pancreas. After receiving symptomatic treatment and a Sippy diet, he was prepared for operation by daily blood transfusions and administration of vitamin K. On Nov. 14, an exploratory celiotomy disclosed that the stomach was diffusely infiltrated by a carcinoma, invading the lesser omentum and regional and portal lymph nodes. There were also neoplastic nodules in the liver and on the inferior surface of the diaphragm. Biopsy specimens were secured from a lymph node about the lesser curvature of the stomach and from the diaphragm, the liver, and the skin. Microscopic examination revealed neoplastic columnar cells in acinar tubular structures in the lymph node and in lymphatic channels of the diaphragm. In the liver, there were bile pigment granules in the cells near the central vein and cords of neoplastic cells in lymph spaces. The skin biopsy revealed acanthosis nigricans (Fig. 7). Postoperatively the patient rallied, but the course remained downhill and he died on Dec. 2.

At necropsy the body was 165 cm. long, weighed 115 lb. (52.1 kg.), and was emaciated. There was marked icterus. The skin was dark-brown and

Fig. 3.—Acanthosis nigricans of the axilla.

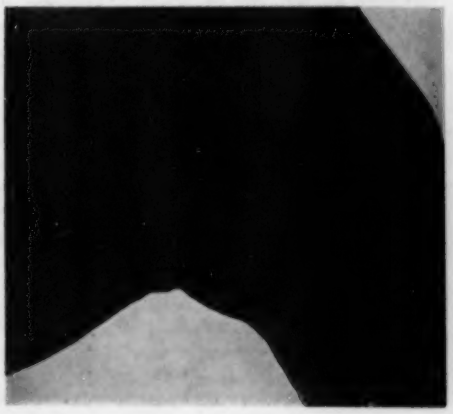


Fig. 4.—Carcinoma of the stomach in a patient with acanthosis nigricans. Neoplastic nodules protrude on the serosal surface. Lymph nodes along the lesser curvature are enlarged by neoplastic involvement.

leathery. In addition, in areas it was coarsely velvety and still darker, with closely packed projections 0.2 to 0.5 cm. wide and elevated 0.2 cm. They were symmetrically distributed over the chin, around the nipples, and in the pubic region. The skin of the axillae had taller projections and deeper crevices (Fig. 3). The anterior surface of the forearms, the dorsa of the hands and fingers and the knees, and the dorsa of the feet were similarly involved. The palms and soles were gray-brown with the skin thickened. Black warts were on the nose, on the right forearm, and on both hands. The surface of the tongue was coarsely velvety. The mucosa of the soft palate and of the peritonsillar areas was light-gray; that of the hypopharynx, white.

The peritoneal cavity was free of fluid. The liver edge extended 15 cm. below the xiphoid process and was bound to the abdominal wall, to the mesocolon, and to the diaphragm by neoplastic tissue. Tumor nodules were scattered over the diaphragmatic surface of the right pleural cavity and over the left visceral pleura. There was no excess fluid in the pericardial cavity, and no change was noted in the heart.

The liver weighed 2200 gm. About one-tenth of the parenchyma was replaced by neoplastic tissue, with the main mass, 3×3 cm., at the porta, extending along the bile ducts. The hepatic ducts and cystic duct were embedded in tumor tissue. The gall bladder contained no concretions. The common bile duct was infiltrated with tumor tissue in its proximal portion and was compressed by enlarged lymph nodes infiltrated with neoplastic tissue. The wall of the esophagus in its midportion contained an oval gray nodule, 2×0.8 cm. The stomach was bound to the liver by neoplastic tissue along the lesser curvature. About one-third of the stomach wall was replaced by neoplastic tissue, forming cauliflower-like projections elevated 3 cm. There were scattered tumor implants on the serosa and in the wall of the duodenum (Fig. 4). Innumerable neoplastic nodes were in the omentum and mesentery. A neoplastic mass, 4×4 cm., separated the rectum from the urinary bladder and surrounded the lower portions of the ureters and seminal vesicles. No changes were noted in the spleen and pancreas. The kidneys weighed 300 and 230 gm. The right adrenal gland weighed 7 gm. and the left 10 gm. The cortex was 0.1 to 0.2 cm. thick, gray-white, and devoid of yellow tinge.

The sella turcica was enlarged to 1.3×1.7 cm., and in the dura covering it there was an opening 1.2×1.5 cm. (Fig. 5). The anterior clinoid processes were hard; the lateral and posterior walls of the sella were fibrous and elastic; the floor and anterior wall were bony and thin (Fig. 6).

Fig. 5.—Spacious opening in the diaphragm of the sella turcica. Beneath is a cystic space pressing the hypophysis against the floor.

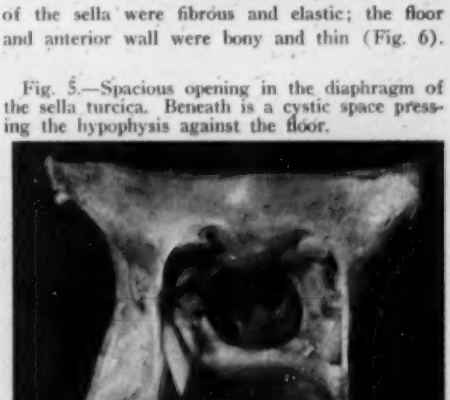


Fig. 6.—Roentgenograms of the sella turcica outlining the space. The dorsum sellae is thinned.

The stalk of the hypophysis was 1.5 mm. wide and 1 cm. long. The hypophysis was flattened against the floor of the sella and was 1.2×1×0.3 cm. The center was thinner than the sides, which formed a U-shaped ridge, to which the stalk was attached posteriorly. The hypophysis weighed 330 mg. (about half the normal). The brain weighed 1300 gm. Cut surfaces disclosed an apparent decrease of the nerve substance and slight enlargement of the lateral and third ventricles. The pineal body was a thin-walled sac, 1×0.8×0.6 cm., containing clear fluid.

Microscopic studies of the skin revealed similar appearance to that of the portion obtained at operation (Fig. 7). The surface was wrinkled, with coarse projections. The epithelium was of fairly even width, with elongated, pointed, or broad epithelial ridges extending into the corium. The cells of the basal layer were packed with dark-brown or black granules. The spinous layer was four to six cells deep, with intercellular bridges easily made out. The granular layer was delicate, and the anuclear keratinized layer was broad, with sheets of keratin filling the spaces between the wrinkles and covering the crests of the elevations. No remarkable change was noted in the corium. In the stomach (Fig. 8) neoplastic columnar cells formed acinar tubular structures, replacing the mucosa and infiltrating tissue spaces and lymph channels that penetrated the muscularis mucosae, the submucosa, and the muscle coats. The neoplastic cells produced the same over-all pattern at all sites of metastasis. In the tongue, coarse papillary projections were covered by a fairly broad layer of stratified squamous epithelium, with elongated, anastomosing epithelial ridges extending into the basal layer; intercellular bridges were conspicuous in the spinal layer, and in the keratinized layer cell nuclei were abundant. The oval mass in the wall of the esophagus was a leiomyoma. The hypophysis had the usual pattern in its glandular, intermediate, and nervous portions. No relevant changes were noted in any of the other organs examined, including the adrenal glands and the thyroid.

Fig. 7.—Microscopic appearance of the skin with acanthosis nigricans. Reduced to 76% of mag. × 100.

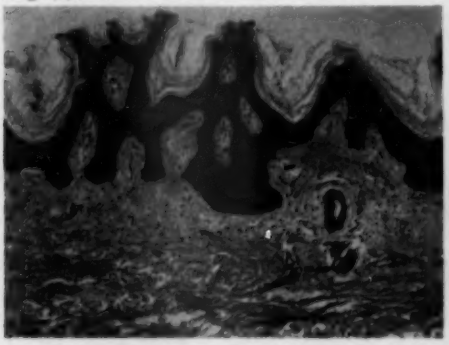
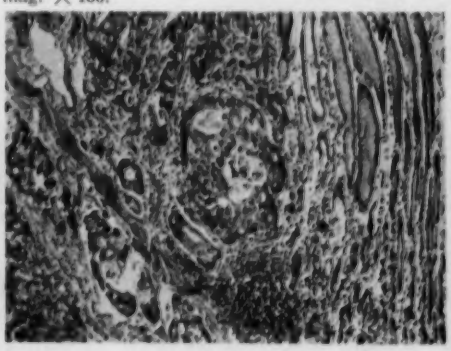


Fig. 8.—Microscopic appearance of the carcinoma shown in Figure 4. Reduced to 76% of mag. × 100.



## Comment

The significance of both types of acanthosis nigricans and the biologic problem they present have been analyzed by Curth, whose detailed investigation renewed interest in the subject.[2] In our case no pertinent information was provided concerning the possible genetic factors considered by Curth. The acanthosis nigricans became manifest about the time that the cancer started producing symptoms.

The cyst in the stella turcica appeared to be an expansion of the subarachnoid space through a defect in the diaphragm of the sella. Endocrine or sympathetic factors have been considered in the etiology of acanthosis nigricans because of the remote analogy with the pigmentation in Addison's disease. In our case, despite the anomaly and relative atrophy of the hypophysis, there were no noticeable signs of hypophysial or adrenal dysfunction. Whether the incidental observation of the cyst in the sella turcica is of any significance remains problematical.

## Summary

Acanthosis nigricans is recorded in a Negro man, age 55, who died of carcinoma of the stomach and in whom at necropsy a cyst of the hypophysial region was observed.

Veterans Administration Hospital, 2002 Holcombe Blvd. (31).

## REFERENCES

1. Curth, H. O.: Acanthosis Nigricans and Its Association with Cancer, Arch. Dermat. & Syph. 57:158-170, 1948.

2. Curth, H. O.: Significance of Acanthosis Nigricans, A. M. A. Arch. Dermat. & Syph. 66:80-100, 1952.

# Necrotizing Vascular Disease in Rat

## *II. Role of Sodium Chloride*

**SIMON KOLETSKY, M.D., Cleveland**
**With the Technical Assistance of Carol Van Rooy and Annette Press**

Injury to the rat kidney often leads to necrotizing vascular disease and hypertension. It was suggested in a previous report [1] that the vascular change might result from a renal dysfunction associated with imbalance of electrolytes in the tissues. The present study, dealing with the effect of salt on development of the vascular disease, supports this concept. While emphasis is primarily directed to the vascular lesions, many of the observations also apply to hypertension.

### Method

Young adult white male rats, weighing about 200 gm., were used. Three different techniques were employed to induce hypertensive vascular disease: (1) bilateral renal artery ligation (ligation of the anterior branch of the left renal artery and of the main right renal artery); (2) unilateral renal artery ligation (ligation of the anterior branch of the left renal artery with the right kidney intact), and (3) a figure-of-eight ligature applied to the left kidney and resection of the right kidney.

The ligation procedures were carried out as previously described.[1] The figure-of-eight ligature [2] was snug, rather than tight, and did not produce infarction.

The rats operated on were subjected to three different dietary regimes, as follows: (1) a standard diet consisting of Chow and tap water for drinking, (2) a salt diet composed of Chow with 1% sodium chloride substituted for the drinking water, and (3) a salt-free diet * with tap water for drinking.

Submitted for publication Nov. 12, 1956.

From the Institute of Pathology, Western Reserve University School of Medicine.

Supported by grants from the Cleveland Area Heart Society and the U. S. Public Health Service.

* Composition: casein, 18%; sucrose, 72%; corn oil, 5%; vitamin fortification; Na-free salt mixture, 5%. The salt mixture consisted of calcium carbonate, 37.5%; monobasic potassium phosphate, 28.4%; potassium chloride, 18.9%; ferric citrate, 9.5%; magnesium oxide, 3.8%, and copper sulfate, 1.9%.

The salt diet was begun immediately after surgery and was continued until the time the animal was killed. The animals drank from 20 to 90 cc. of 1% NaCl daily. The salt-free diet was instituted from two to four weeks prior to operation and then continued after surgery until the time of killing the animal. All diets were offered ad libitum.

A group of 16 rats with bilateral renal artery ligation was placed on each of the three dietary regimes. The same number of rats with unilateral renal artery ligation and with the figure-of-eight renal ligature were also placed on each of the diets. Thus, there was a total of 144 experimental rats.

Surgery was performed with clean technique under pentobarbital sodium anesthesia. The procedures were completed in one stage.

Blood pressure was measured by the plethysmographic technique. Readings were made daily for a period of two to four days before surgery and at least once daily after surgery. All animals were killed seven days after surgery. Every rat was autopsied, and microscopic sections were prepared from various organs.

### Results

*Vascular Necrosis.*—Table 1 shows the influence of dietary salt on the development of vascular necrosis following different forms of renal injury.

Renal Artery Ligation: All rats on standard diet subjected to either bilateral or unilateral renal artery ligation developed necrotizing vascular disease. The lesions were more pronounced in the group with bilateral ligation. The same universal incidence was obtained with a salt diet except that the vascular change was often augmented in severity. With a salt-free regime all but one animal with bilateral renal artery ligation showed necrosis of vessels, whereas the incidence of vascular disease in the unilaterally ligated group was reduced from 100% to 50%.

TABLE 1.—*Role of Salt in Vascular Necrosis*

| Renal Injury | Vascular Necrosis, % Incidence | | |
|---|---|---|---|
| | Salt Diet | Standard Diet | Salt-Free Diet |
| Renal artery ligation, bilateral | 100 | 100 | 93 |
| Renal artery ligation, unilateral | 100 | 100 | 50 |
| Figure-of-eight ligature, nephrectomy | 100 | 38 | 0 |

Figure-of-Eight Renal Ligature: Thirty-eight per cent of animals on standard diet had vascular lesions. When 1% sodium chloride solution was substituted for the drinking water, the incidence rose to 100%, and, conversely, on a salt-free diet no vascular necrosis was observed in any of the animals.

The vascular lesions were usually of the polyarteritis nodosa type. They involved small arteries and arterioles and were observed only microscopically, most frequently in mesentery and pancreas but also in gastrointestinal tract, heart, adrenals, liver, and kidneys. The proliferative and exudative components varied in degree, and sometimes the lesions were essentially of the pure necrotizing type, especially in organs like the heart and kidney.

Another fairly common microscopic lesion was focal myocardial necrosis. In general this followed the same pattern of incidence in relation to dietary sodium chloride as the vascular disease.

*Hypertension.*—The influence of salt on the development of hypertension in rats subjected to renal injury is shown in Table 2. With the standard diet used as a basis for comparison, the effect of increased salt intake was seen to be promotion of hypertension, while a salt-free regime in general tended to retard elevation of blood pressure.

TABLE 2.—*Role of Salt in Hypertension*

| Renal Injury | Hypertension* | | |
|---|---|---|---|
| | Salt Diet | Standard Diet | Salt-Free Diet |
| Renal artery ligation, bilateral | + | + | + or 0 |
| Renal artery ligation, unilateral | + | + or 0 | 0 |
| Figure-of-eight ligature; nephrectomy | + | + or 0 | 0 |

* + indicates hypertension; 0, no hypertension; + or 0, some rats hypertensive, others normotensive.

Bilateral Renal Artery Ligation: All rats on the standard diet showed a gradual elevation of blood pressure to levels of about 160-170 mm. Hg at one week after operation. The results were similar for animals drinking 1% sodium chloride, and no tendency to higher pressures was detected. With a salt-free diet, however, 7 of the 16 rats had no significant change in blood pressure after surgery, while the rest had an elevation to about 160 mm. Hg.

Unilateral Renal Artery Ligation: With the standard diet most animals showed a slight elevation of blood pressure to about 150 mm. Hg after one week. However, several had either no rise or an equivocal one. On the salt regime all animals had a rise in pressure, usually to about 160 mm., and in a few instances to 180 mm. Hg. In contrast, rats on a salt-free regime generally remained normotensive. Only three animals could be judged hypertensive.

Figure-of-Eight Renal Ligature: Half the animals on the standard diet showed a slight trend toward higher blood pressure after the operative procedure, but in no instance did the level exceed 150 mm. Hg. The rest were normotensive. Almost all rats on 1% sodium chloride solution for drinking fluid had elevated pressures with readings of 150-160 mm. Hg at seven days. Conversely, none of the animals on the salt-free regime had a rise in pressure.

No clear correlation between hypertension and vascular necrosis was obtained. Although most of the animals with hypertension also had necrotizing vascular disease, many rats with vascular lesions were normotensive, especially those placed on a salt-free diet and subjected to bilateral or unilateral renal artery ligation.

## Comment

Renal injury constitutes the principal method for the rapid induction of vascular necrosis in the rat. For example, renal artery ligation yields lesions in a few days.[1]

Other methods, such as high intake of salt in the diet [3,4] or administration of steroids,[5] while effective, require weeks or months. Necrotizing vascular disease can also occur rapidly after total nephrectomy [6,7] (renoprival vascular disease), but very little work in this area has been done on the rat as compared with the dog.

Of the techniques used in the present study, bilateral renal artery ligation resulted in the most pronounced vascular lesions. Unilateral artery ligation was next in effectiveness and gave a much higher incidence of vascular disease than the figure-of-eight method. The reason for this is obscure. Since the ligation technique involved only one kidney, reduction in renal mass was apparently not a critical factor. Perhaps the explanation lies in the different type of renal injury, i. e., infarction versus ischemia, caused by these procedures.

Elevation of blood pressure and vascular change often seem to evolve simultaneously after renal injury. However, there is no obligatory connection, and either can occur independently of the other. In a previous report [1] evidence was presented that the vascular lesions are not caused by hypertension, as many have claimed. Neither are they due, in all probability, to renal excretory failure, infection, or an allergic state. The fact that the same type of vascular necrosis follows total nephrectomy is against a necrotizing agent elaborated by the injured kidney as the causative factor, but does not finally exclude such a factor.

In the rat there is no doubt that sodium chloride is important in the pathogenesis of some forms of hypertensive vascular disease, such as those following high intake of salt or the administration of steroids. However, whether it has a significant connection with renal or with renoprival hypertensive vascular disease is uncertain. In the dog, salt appears to be a conditioning factor in the renoprival disease,[8] along with other items, such as the dietary protein level.[8,9]

There is good evidence that additional salt in the diet aggravates the vascular disease following kidney damage. Chute and others [10] regarded the vascular lesions which developed in alloxan-treated rats as due to the action of salt in the presence of damaged kidneys. From the use of various diets Race and Peschel [11] reported that vascular disease was caused by a combination of renal injury and feeding of NaCl. The results obtained by Kempner and associates [12] supported this conclusion.

It should be emphasized that renal injury does not require exogenous salt loading in order to produce vascular lesions. The latter will develop consistently a few days after renal artery ligation in rats on a standard diet. However, as shown in this study, increased intake of NaCl following ligation served to enhance the vascular lesions in number and distribution. On the other hand, complete withdrawal had the opposite effect, and in fact the lesions were eliminated entirely in some animals by a salt-free regime.

The relation of vascular necrosis to salt intake was perhaps most clearly evident when the figure-of-eight kidney ligature served as the mode of renal damage. With this technique vascular lesions occurred in 38% of animals which were maintained on a standard diet. The incidence rose to 100% when NaCl was added to the drinking fluid, and, conversely, a salt-free diet practically abolished the lesions.

Such observations constitute strong evidence that NaCl enters into the chain of events between renal injury and the development of vascular necrosis. There is also some indication that the salt complements renal injury in the production of vascular necrosis, since the need for exogenous salt seems to vary inversely with the severity of the renal damage. Thus, high salt intake in conjunction with mild renal injury, i. e., figure-of-eight ligature, readily causes disease of vessels in animals which would show no lesions if kept on a standard diet.

Also, vascular lesions can develop even in animals with drastic restriction of salt, provided the renal injury is severe, i. e., bilateral renal artery ligation. The circumstance of a salt-free regime is not interpreted as excluding NaCl from participation in pathogenesis, since it is available from endogenous source.

The precise mechanism by which NaCl influences the development of vascular disease and hypertension following renal injury is obscure. However, this behavior of the salt is in keeping with the concept that hypertensive vascular disease may be related to a renal dysfunction in the handling of electrolytes, which, in turn, leads to intracellular imbalance of electrolytes, especially Na and K, in the tissues. According to this view, hypertension results from increased vascular tonus initiated by changes in the distribution and metabolism of salt and water, and vascular necrosis is attributed to a critical upset in cellular electrolytes. Tobian and Binion [13] cite studies on the kinetics of actomyosin which indicate that altered composition of electrolytes might have important effects on the tension of arterial smooth muscle.

That tissue necrosis may be caused by electrolyte imbalance is well illustrated by the potassium-deficiency state.[14-17] Under such a regime rats develop myocardial necrosis (but apparently not vascular necrosis), which resembles that observed in hypertensive vascular disease. The necrosis is associated with reduction in the potassium content of the heart and increase in the sodium content. There is no clear notion as to just how the electrolyte upset in potassium-deficient animals results in tissue death. Perhaps the same mechanism is operative here as for the vascular necrosis following renal injury. French [17] speculated that electrolyte imbalance might destroy cellular integrity through alteration in the physical state of the actomyosin complex or by interference with the energy-producing mechanism of the cell. Both of these are dependent on, or are influenced by, the presence and/or relative concentration of the alkaline ions. As French pointed out, the mechanism is probably not a simple one, since the degree of necrosis is significantly influenced by a number of items, such as salt intake, acid-base balance, and adrenocortical hormones.

It has been shown that myocardial necrosis in potassium-deficient rats is absent [18] or minimal [19] with low salt intake and marked with high salt intake.[19] This suggested to Cannon, Frazier, and Hughes that replacement of K by Na might have a toxic effect on intracellular metabolic processes. According to Cooke et al.,[20] the intracellular exchange of Na and K in the potassium-deficient state is probably also associated with exchange of H ions in order to provide electrical neutrality. Hence, it may be that accumulation of H ions with increased cellular acidity, together with accumulation of sodium, initiates the necrotizing process.

Several authors have studied electrolyte changes in the tissues of animals with hypertensive vascular disease. This concerned mainly alterations in sodium, potassium, and water content in both intracellular and extracellular phases. The earlier studies emphasized increased sodium and chloride content of muscle and a decrease in potassium.[21,22] In rats rendered hypertensive by the figure-of-eight technique, Greene and Sapirstein [23] found a considerable increase in total body sodium, possibly due to intracellular accumulation of sodium, but no corresponding fall in potassium, as in DCA (desoxycorticosterone acetate) hypertension. With the same technique, Tobian and Binion [13] reported an increase in both intracellular sodium and potassium in the aorta, whereas skeletal muscle and brain showed no significant change. With DCA hypertension there was an increase in sodium and a decrease in potassium in skeletal muscle, while both sodium and potassium were elevated in the brain. Thus, the results appear to vary both with the

technique employed to induce hypertension and with the type of tissue analyzed.

On the other hand, Ledingham,[24] utilizing the renal artery clip technique in rats, concluded that expansion of the extracellular fluid could account for the increase in sodium and the decrease in potassium concentration in hypertension of short duration. He doubted that there was a significant alteration in intracellular sodium and potassium. Grollman[25] analyzed electrolytes in several organs, including heart and skeletal muscle of choline-deficient rats, and found no significant difference in composition in the hypertensive animals as compared with normal controls.

Perhaps the variable results in these studies stem in part from difference in experimental design. Aside from this, investigation of electrolytes is hampered by some uncertainty about procedures, i. e., whether the methods for extracellular fluid space are accurate and whether assumptions made in order to calculate electrolyte partition are valid. The available data indicate an association between hypertensive vascular disease and electrolyte alterations in tissues but do not prove that a causative relationship exists. The electrolyte changes might be secondary to other tissue alterations associated with hypertensive vascular disease or might result merely from renal injury and be incidental to the hypertension.

The fact remains, however, that the production of vascular disease and hypertension through kidney damage is clearly influenced by NaCl. The presence or absence of lesions, even on a short-term basis, can be shown to depend on the availability of salt. Such behavior of NaCl, even though unexplained, favors the view that hypertensive vascular disease results from imbalance and faulty metabolism of electrolytes brought about by renal injury. An electrolyte upset would be aggravated by salt loading and lessened by its withdrawal. However, the mode of action of NaCl obviously requires further clarification. In this regard, items like the handling of sodium following renal injury and alterations in tissue electrolytes are potentially important and deserve further study.

## Summary

Hypertensive vascular disease was induced in the rat by means of renal artery ligation and by the figure-of-eight kidney ligature. It was found that the development of both vascular lesions and hypertension were critically influenced by the amount of dietary salt. Increased intake of NaCl served to enhance the disease, whereas restriction had the opposite effect.

There was some indication that the NaCl served to complement renal injury in the production of vascular disease. Mild injury plus high salt intake yielded lesions which would not occur if the salt intake were normal or low. Severe kidney damage induced lesions in spite of drastic salt restriction.

The significant role of NaCl in the production of vascular lesions suggests that the latter might develop through an imbalance of cellular electrolytes in the tissues.

Institute of Pathology, Western Reserve University School of Medicine.

## REFERENCES

1. Koletsky, S.: Necrotizing Vascular Disease in Rat: I. Observations on Pathogenesis, A. M. A. Arch. Path. 59:312-320, 1955.

2. Grollman, A.: A Simplified Procedure for Inducing Chronic Renal Hypertension in the Mammal, Proc. Soc. Exper. Biol. & Med. 57:102-104, 1944.

3. Gepts, W.: Lésions de myocardite, de périartérite et de pancréatite provoquées chez des rats soumis à un régime surchargé en chlorure de sodium, Compt. rend. Soc. biol. 146:307-309, 1952.

4. Toussaint, C.; Wolter, R., and Sibelle, P.: Effets de l'ingestion de grandes quantités de chlorure de sodium chez le rat, Compt. rend. Soc. biol. 147:1637-1639, 1953.

5. Selye, H., and Pentz, E. I.: Pathogenetical Correlations Between Periarteritis Nodosa, Renal Hypertension and Rheumatic Lesions, Canad. M. A. J. 49:264-272, 1943.

6. Kolff, W. J., and Fisher, E. R.: Pathologic Changes After Bilateral Nephrectomy in Dogs and Rats, Lab. Invest. 1:351-363, 1952.

7. Koletsky, S.: Unpublished data.

8. Muirhead, E. E.; Jones, F., and Graham, P.: Hypertension in Bilaterally Nephrectomized Dogs in Absence of Exogenous Sodium Excess, A. M. A. Arch. Path. 56:286-292, 1953.

9. Kolff, W. J., and Page, I.: Influence of Protein Diets on Development of Renoprival Hypertension in Dogs, Am. J. Physiol. 181:580-584, 1955.

10. Chute, A. L.; Orr, J. L.; O'Brien, M. J., and Jones, E. E.: Vascular Lesions in Alloxan Diabetic Rats, A. M. A. Arch. Path. 52:105-114, 1951.

11. Race, G. J., and Peschel, E.: Pathogenesis of Polyarteritis Nodosa in Hypertensive Rats, Circulation Res. 2:483-487, 1954.

12. Kempner, W.; Peschel, E., and Black-Schaffer, B.: Effect of Diet on Experimental Hypertension and on the Development of Polyarteritis Nodosa in Rats, Circulation Res. 3:73-78, 1955.

13. Tobian, L., Jr., and Binion, J.: Artery Wall Electrolytes in Renal and DCA Hypertension, J. Clin. Invest. 33:1407-1414, 1954.

14. Schrader, G. A.; Prickett, C. O., and Salmon, W. D.: Symptomatology and Pathology of Potassium and Magnesium Deficiencies in the Rat, J. Nutrition 14:85-109, 1937.

15. Follis, R. H., Jr.; Orent-Keiles, E., and McCollum, E. V.: Production of Cardiac and Renal Lesions in Rats by a Diet Extremely Deficient in Potassium, Am. J. Path. 18:29-39, 1942.

16. Smith, S. G.; Black-Schaffer, B., and Lasater, T. E.: Potassium Deficiency Syndrome in the Rat and in the Dog: Description of the Muscle Changes in the Potassium-Depleted Dog, Arch. Path. 49:185-199, 1950.

17. French, J. E.: A Histological Study of the Heart Lesions in Potassium-Deficient Rats, A. M. A. Arch. Path. 53:485-496, 1952.

18. Freed, S. C., and Friedman, M.: Depressor Effect of Potassium Restriction on Blood Pressure of the Rat, Proc. Soc. Exper. Biol. & Med. 78:74-77, 1951.

19. Cannon, P. R.; Frazier, L. E., and Hughes, R. H.: Sodium as a Toxic Ion in Potassium Deficiency, Metabolism 2:297-312, 1953.

20. Cooke, R. E.; Segar, W. E.; Cheek, D. B.; Coville, F. E., and Darrow, D. S.: The Extrarenal Correction of Alkalosis Associated with Potassium Deficiency, J. Clin. Invest. 31:798-805, 1952.

21. Eichelberger, L.: Distribution of Water and Electrolytes Between Blood and Skeletal Muscle in Experimental Hypertension, J. Exper. Med. 77:205-213, 1943.

22. Laramore, D. C., and Grollman, A.: Water and Electrolyte Content of Tissues in Normal and Hypertensive Rats, Am. J. Physiol. 161:278-282, 1950.

23. Greene, R. W., and Sapirstein, L. A.: Total Body Sodium, Potassium and Nitrogen in Rats Made Hypertensive by Subtotal Nephrectomy, Am. J. Physiol. 169:343-349, 1952.

24. Ledingham, J. M.: Distribution of Water, Sodium and Potassium in Heart and Skeletal Muscle in Experimental Renal Hypertension in Rats, Clin. Sc. 12:337-349, 1953.

25. Grollman, A.: The Water and Electrolyte Content of the Tissues in Hypertension, Circulation Res. 2:541-543, 1954.

# Allergic Granuloma

*A Case of Loeffler's Syndrome with Verrucous Endocarditis*

**HENRY B. MARSDEN, M.B., D.C.H., D.Path., and JOHN K. MORGAN, M.D., M.R.C.P., Pendlebury, Lancs., England**

Granulomatous lesions in the lungs have been described by Ehrlich and Romanoff [1] in patients showing such evidence of allergy as, for example, eosinophilia, asthma, and hay fever. In addition, granulomata in the upper and lower respiratory tract in association with necrotizing angiitis and glomerulitis have been reported by Godman and Churg [2] and Walton and Leggat.[3] Lesions involving the mitral valve were found in two of the cases of Godman and Churg,[2] and in a third case mural thrombi were present in the heart.

We have investigated a patient with disseminated visceral granulomatosis and extreme eosinophilia. The presence of mitral vegetations and glomerular scarring, together with certain facts which may throw some light on the possible etiology of the lesions, prompted this report.

## Report of Case

### *History*

A married woman, aged 42, was referred to the Manchester and Salford Hospital for Skin Diseases in 1953 from a tuberculosis clinic elsewhere. She had received treatment for a patch of lupus vulgaris since the age of 3 years. Examination showed a typical area of lupus vulgaris over the posterior aspect of the left thigh, the diagnosis being confirmed by biopsy. Treatment with curettage and streptomycin was given, but the latter drug had to be discontinued almost at once, owing to the development of a generalized skin eruption.

There was rapid healing at the operation site, but eight months later discrete firm red nodules, 3-5 mm. in diameter, developed over the back, chest, neck, and forehead. Biopsy of one of the nodules confirmed the diagnosis of dissemination of lupus vulgaris. The patient was seen at the outpatient department eight months after this incident owing to breaking down of the scar with ulceration at the site of the original lesion on the left thigh. An x-ray of the chest showed no abnormality, and treatment with amino salicylic acid (PAS) and isoniazid was given.

The response to treatment was unsatisfactory, and admission to hospital was advised. Examination at this time showed scarring over the posterior aspect of the left thigh with numerous surrounding nodules. There was an ulcer at the inner edge of the scar about 3.5 cm. in diameter and 4 cm. in depth, with thick slough over the base. The discrete nodules on other parts of the body, mentioned earlier, were still present.

The patient was transferred to a general hospital elsewhere, where the ulcer was excised; but the wound broke down and reexcision had to be carried out on several occasions. The histological report at this time was of tuberculous granulation tissue.

One year later, in 1955, the patient was readmitted to the Manchester Hospital for Skin Diseases because of areas of continuing ulceration in the operation scar. Biopsy specimens from two such areas now showed a change in the histological picture, with foci of necrosis in the dermis, packed with degenerate eosinophilic leukocytes. The surrounding infiltration was mainly lymphocytic in type, with large mononuclear cells, some of which had a bizarre nuclear appearance and others displayed mitotic figures. The white blood cells at this time numbered 56,000 per cubic millimeter, with 59% eosinophilic leukocytes. An x-ray examination of the chest revealed the presence of multiple rounded opacities (Fig. 1).

The patient's condition deteriorated without remission in spite of treatment with antibiotics and corticotropin. Splinter hemorrhages under the nails were noted some weeks later, although no cardiac murmur was detected. There were mental confusion and quadriplegia prior to death, in coma, two months after the last admission. The white blood cell count had risen at one point to 93,600 cells, with 62% eosinophils, but terminally showed 10,600 cells with 37% eosinophils.

Submitted for publication Aug. 29, 1956.

From the Manchester and Salford Hospital for Skin Diseases.

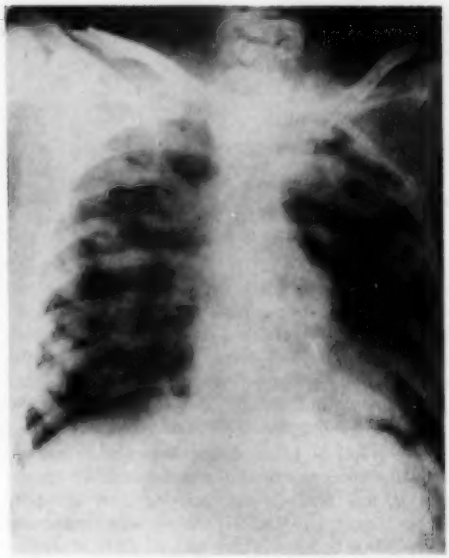


Fig. 1.—Radiograph of the chest showing rounded areas of infiltration in the lung fields.

### *Postmortem Examination*

Summary of findings: Granulomata in the lungs, kidneys, pancreas, and thyroid; verrucae on the mitral valve with interstitial scarring in the myocardium and mural thrombi in the left ventricle; fibrosis of renal glomeruli; small necrotic foci in the brain stem, probably the result of emboli.

Gross Appearance: Heart. Small scars were seen over the epicardial surface. The leaflets of the tricuspid valve were thickened, but the pulmonary and aortic valves were not remarkable. The mitral valve showed marked thickening with shortening of the chordae tendineae. A row of brown-yellow nodules, 2-3 mm. in diameter, was seen along the contact line of the valve, with no involvement of the free margin. Large "pocket" lesions were present between the valve and the ventricular wall, and there were mural nodules in the left ventricle with considerable thinning of the wall near the apex of the chamber (Fig. 2).

Lungs. There were bilateral pleural adhesions and areas of consolidation. White nodules were scattered throughout each lung, with no particular distribution. These nodules were firm, roughly spherical, and up to 3 cm. in diameter. They showed small necrotic cores and were sharply demarcated from the surrounding tissue (Fig. 3).

Kidneys. The organs were slightly shrunken, with small depressed subcapsular scars. A white nodule similar to those seen in the lungs was present at the lower pole of the left kidney. There were a few small nodules in the right kidney. The width of the cortex was markedly diminished, and there was some loss of normal vascular markings.

Other organs. A firm, rounded white nodule was present in the body of the pancreas, and a similar lesion was detected in the right lobe of the thyroid gland.

Histology: The structure of the nodules was essentially similar throughout, with the

Fig. 2.—The heart opened to show the left ventricle. The mitral valve is thickened, and vegetations are present, together with "pocket" lesions. Mural thrombi can be seen, and the thickness of the wall is reduced near the apex.

Fig. 3.—Cut surface of lung showing scattered, sharply defined white nodules.

exception of the one in the thyroid, which was entirely amorphous. They showed sharply defined central cores, which consisted of partially destroyed eosinophilic leukocytes with abundant Charcot-Leyden crystals (Fig. 4). In the surrounding tissues extensive fibrosis was present, with large mononuclear or reticulum cells, which sometimes showed large, bizarre nuclei. There were very occasional giant cells, containing three or four nuclei. Vascular involvement, consisting of thrombosis with organization and canalization, was confined to the areas of granuloma formation.

In the heart, the nodules along the mitral valve had an organized hyaline structure with no cellular infiltration apart from the basal portions, where scanty histiocytes and macrophages containing golden pigment were noted. The "pocket" lesions were made up of tangled masses of fibrin, but large numbers of degenerating eosinophils could be seen in some peripheral areas. Fibrin without eosinophils was seen in the mural nodules, but here the underlying cellular reaction was more marked, with lymphocytes, plasma cells, and numerous pigment-laden macrophages. Small scars were present in the myocardium, with some lymphocytic infiltration and moderate amounts of pigment.

In the lungs the alveoli surrounding the granulomata showed hemorrhage with extensive deposition of fibrin. There was fibrosis with organization of alveolar masses, giving rise to Masson bodies.

The kidney showed changes apart from the presence of granulomata. All degrees of glomerular sclerosis, from capsule fibrosis to complete conversion into balls of hyaline fibrous tissue, were noted. In addition, there appeared to be an increase of interstitial connective tissue.

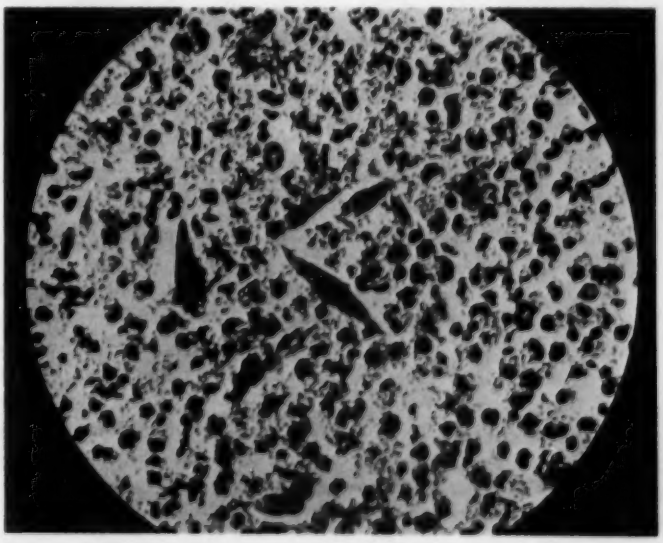


Figure 4.—Microscopic appearance of the center of a granulomatous area, showing necrotic eosinophilic leukocytes and Charcot-Leyden crystals. Hematoxylin and azo-eosin stain; high-power magnification.

The liver had very prominent fatty vacuolation of parenchymal cells, and there was an increase of periarteriolar fibrous tissue in the spleen. Small areas of rarefaction, with macrophage activity, were present in the brain stem. No other organ or tissue examined presented any unusual features, and the marrow showed little increase of eosinophilic leukocytes.

*Bacteriology*

Cultures taken from the lung yielded a growth of Staphylococcus pyogenes and Proteus vulgaris. Microscopic examination and culture failed to reveal the presence of tubercle bacilli in the granulomata.

## Comment

There has been considerable evidence to support the suggestion that an allergic mechanism is involved in the production of granulomata similar to those which have been described in this case. The presence of eosinophilia is significant, and an association with asthma, urticaria, and hay fever has been found. In this connection, it has been shown by Goddard [4] that the formation of granulomata in addition to necrosis of tissue may be seen in hypersensitivity. More recently, von Albertini,[5] investigating antigen-antibody reactions in animals, has produced tissue necrosis followed by a histiocytic reaction with occasional giant cells which were not of the Langhans type.

The agent involved in the production of the hypersensitive state probably varies from case to case. Parasites and pollens have been incriminated, and the presence of disseminated granulomatous lesions in cases showing reaction to sulfonamides has been described by More, McMillan, and Duff.[6] In addition, bacterial products, as suggested by Zuelzer and Apt,[7] appear to play an important part.

Loeffler's syndrome, or the presence of pulmonary infiltration with blood eosinophilia, has been regarded by Ehrlich and Romanoff [1] as too narrow a concept, and this view is supported by the widespread findings in our case. The possibility of the involvement of an allergic mechanism in the production of this syndrome has, however, received strong support by Bayley, Lindberg, and Baggenstoss.[8]

Loeffler thought that tuberculosis was the commonest cause of the syndrome to which his name is now attached, and the opinion was supported by Leitner (cited by Hedvall [9]). This does not now appear to be the case, and the patients reported by Hedvall [9] had negative findings for tuberculosis. However, the latter author, after weighing the evidence, concludes that the syndrome may be produced by various agents, of which tuberculosis is one.

In our patient it seems probable that the lesion on the back of the thigh was indeed lupus vulgaris. The clinical appearance and histological findings were regarded as being quite typical, although cultures for tubercle bacilli were not made. The giant cells were of the Langhans type, thus differing from those seen in the granulomata which were present at autopsy. In addition, von Albertini [5] states that it has not yet been established that an allergic condition is involved in the production of the tuberculoid reaction. We feel that it is most probable that the abnormal response developed in our patient as the result of a chronic tuberculous lesion which had been present for many years.

The absence of granulomata in the liver in our patient is in contrast with the findings of Zuelzer and Apt [7] in early childhood, where this organ is frequently involved in eosinophilic visceral granulomatosis. These authors suggest that the liver may function as the main shock organ in this age group. Further, although eosinophilia was found in the siblings of several of the patients reported by Zuelzer and Apt,[7] there was no significant increase in the level of eosinophils in blood samples taken from the four children of our patient.

Recurrent infection of the paranasal sinuses was a feature in the childhood cases, and this suggests a possible relationship to the cases in which necrotizing angiitis and glomerulonephritis are associated features.

These cases have been described by Godman and Churg [2] and Walton and Leggat [3] under the title of Wegener's granulomatosis. In two of the patients reported by Godman and Churg [2] there were lesions on the mitral valve, and the presence of glomerular scarring in our patient is another feature in common. The complete absence of vascular damage apart from the granulomatous areas is in marked contrast. However, Loeffler's syndrome appears to include a whole range of conditions, from diffuse eosinophilic pneumonitis without vascular changes to necrotizing eosinophilic granulomata with pulmonary or generalized arteritis.

In conclusion, it is thought that the case presented provides some support for the suggestion by Godman and Churg [2] that Loeffler's syndrome, allergic granuloma, and Wegener's granuloma are related conditions.

## Summary

A case of disseminated visceral granulomata with extreme eosinophilia and cardiac lesions is presented.

The etiology of this condition is discussed, and it is thought possible that the picture may have developed as a result of an abnormal response to a chronic tuberculous infection.

It is felt that Wegener's granuloma, allergic granuloma, and Loeffler's syndrome may all be related disease patterns.

Dr. J. H. T. Davies permitted the publication of this case, and Dr. W. Lee and Mr. R. W. Wyse allowed us to use the earlier clinical records. The photographs were taken by Mr. J. Hodgkinson and Mr. H. E. Ward.

Royal Manchester Children's Hospital.

## REFERENCES

1. Ehrlich, J. C., and Romanoff, A.: Allergic Granuloma of the Lung: Clinical and Anatomic Findings in a Patient with Bronchial Asthma and Eosinophilia, A. M. A. Arch. Int. Med. 87:259, 1951.

2. Godman, G. C., and Churg, J.: Wegener's Granulomatosis: Pathology and Review of the Literature, A. M. A. Arch. Path. 58:533, 1954.

3. Walton, E. W., and Leggat, P. O.: Wegener's Granulomatosis, J. Clin. Path. 9:31, 1956.

4. Goddard, J. W.: Granuloma, a Characteristic "Qualitative" Change in Focal Anaphylactic Inflammation, Am. J. Path. 23:943, 1947.

5. von Albertini, A.: Bedeutung der Allergielehre für die Pathologie, Schweiz. Ztschr. Allg. Path. 17:1, 1954.

6. More, R. H.; McMillan, G. C., and Duff, G. L.: The Pathology of Sulfonamide Allergy in Man, Am. J. Path. 22: 703, 1946.

7. Zuelzer, W. W., and Apt, L.: Disseminated Visceral Lesions Associated with Extreme Eosinophilia, Am. J. Dis. Child. 78:153, 1949.

8. Bayley, E. C.; Lindberg, D. O. N., and Baggenstoss, A. H.: Loeffler's Syndrome: Report of a Case with Pathologic Examination of the Lungs, Arch. Path. 40:376, 1945.

9. Hedvall, E.: Transitory Lung Infiltrations with Blood Eosinophilia, Acta tuberc. scandinav. 16:1, 1942.

# Experimental Xanthomatosis in the Rabbit

CHUN-I WANG, M.D.; LOTTE STRAUSS, M.D., and DAVID ADLERSBERG, M.D., New York

Deposition of cholesterol in the skin, in the joints, and in the bones was occasionally observed in rabbits fed cholesterol for prolonged periods of time.[1-4] Microscopically, these lesions simulated closely those of xanthomatosis in man.[5]

The present report presents a systematic study of experimental xanthomatosis in the rabbit. In includes the incidence, the clinical and the roentgenological manifestations, the associated blood-lipid alterations, and the gross and microscopic changes in the skin, joints, and bones.

## Experimental Method

One hundred twenty male rabbits, "Chinchilla gray" or "Giant Checkers," body weight 2.5-3.0 kg. at the beginning of the experiment, were fed cholesterol-treated Purina Chow for periods varying from two weeks to one year, using the technique previously described.[4] The intake of cholesterol per animal was about 1 gm. per day. Blood specimens were drawn from the marginal ear vein or the central ear artery at intervals of two to four weeks. Plasma cholesterol was determined by the Sperry-Schoenheimer method,[6] phospholipids by Sperry's modification of the Fiske-SubbaRow method,[7] and total lipids by the gravimetric method of Bloor.[8]

Since our primary interest was focused on the early development of aortic atherosclerosis and of xanthomatosis in the skin and joints, about two-thirds of the animals were killed within a period of three months of cholesterol feeding: Of these, 10 were killed after two weeks, 20 after one month, 41 after two months, and 16 after three months. Of the remaining 33 animals, approximately 5 were killed at the end of each subsequent month. All of the organs obtained at autopsy were subjected to extensive pathological studies.[9,10] In addition, a small piece of skin from the abdominal wall and one from the hindleg were taken for microscopic sections. Four untreated animals were used as controls.

## Incidence of Xanthomatosis and Plasma Lipids

No gross xanthomata were noted during the first month of cholesterol feeding. However, a small number of animals (2.5%) exhibited cutaneous xanthomata two to three months after institution of cholesterol feeding. After 3-6 months 21% of the animals, after 7-9 months 50%, and after 10-12 months, 100% showed skin xanthomata (Table).

Although the elevations of the plasma lipids varied considerably from animal to animal, a general trend was noted in that all plasma lipid fractions increased progressively during the first three months of

Submitted for publication Aug. 12, 1956.

Supported in part by Research Grant H-982 from the National Institutes of Health, U. S. Public Health Service.

From the Departments of Medicine and Pathology, Mount Sinai Hospital. Research Assistant in Medicine, Mount Sinai Hospital (Dr. Wang); Associate Pathologist, Mount Sinai Hospital (Dr. Strauss); Associate Attending Physician for Metabolic Diseases, Mount Sinai Hospital (Dr. Adlersberg).

*Skin Xanthoma and Plasma Lipid Partition in Cholesterol-Fed Rabbits*

| | | Duration of Cholesterol Feeding, 1 Gm/day, Mo. | | | |
|---|---|---|---|---|---|
| | Normal | 1-3 | 4-6 | 7-9 | 10-12 |
| No. of animals | 120 | 120 | 33 | 19 | 4 |
| No. with xanthoma (%) | 0 | 3 (2.5%) | 7 (21%) | 10 (50%) | 4 (100%) |
| Cholesterol, total, mg/100 cc. | 50 | 1280 | 1098 | 830 | 787 |
| Esterified, mg/100 cc. | 35 | 972 | 805 | 601 | 543 |
| Phospholipids, mg/100 cc. | 105 | 440 | 450 | 379 | 371 |
| Total lipids, mg/100 cc. | 330 | 2020 | 2465 | 2006 | 2267 |

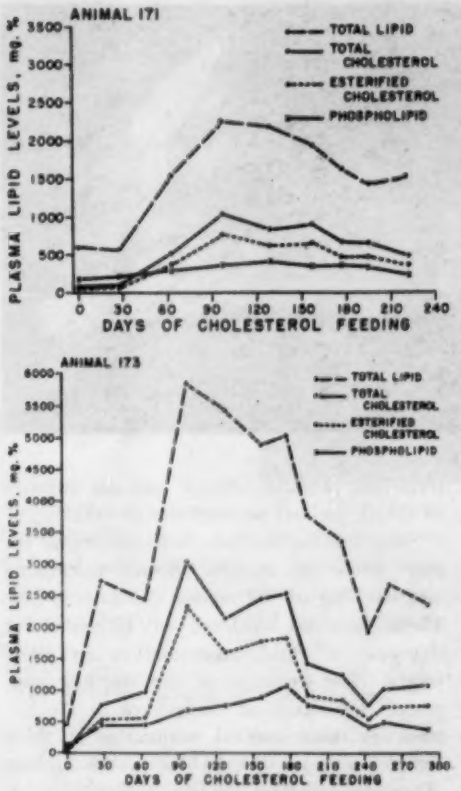



Fig. 1.—Above, plasma lipid levels of Rabbit 171, fed cholesterol-supplemented Chow for 222 days. Note the steady increase of all lipid fractions in the first 90 days, moderate decrease thereafter, and stabilization at lower levels after 180 days. Small nodular skin xanthomata were first noticed after three months of cholesterol feeding. The skin lesions progressed and extended to all four extremities, despite receding plasma lipid levels.

Below, plasma lipid levels of Rabbit 173, fed cholesterol-supplemented Chow for 291 days. Note extremely rapid and marked increase of all plasma lipid fractions during the first 3 months of cholesterol feeding, the subsequent drop of the levels after 4-6 months, and the leveling off at a lower plateau after 7-10 months. Although the plasma lipid levels were exceptionally high in this animal, skin xanthomata were first observed after approximately seven months of cholesterol feeding.

cholesterol feeding.[4] Thereafter, the lipid levels decreased moderately after approximately five to six months and became stabilized at a lower plateau. Figure 1 depicts individual variations of plasma lipid levels in two animals fed simultaneously equal amounts of cholesterol. The Table presents the average values of the plasma lipid fractions for the entire group of animals. The total plasma cholesterol rose from 50 to 1280 mg. per 100 cc. after 1-3 months of cholesterol feeding, and decreased moderately to 1098 mg. after 4-6 months and to 830 and 787 mg. per 100 cc. after 7-9 and 10-12 months, respectively. The percentage of esterified cholesterol remained unchanged. The elevation of plasma phospholipid was far behind that of the cholesterol; it reached a maximum of 450 mg. per 100 cc. at the end of four to six months of cholesterol feeding and remained stabilized at a somewhat lower level for the remaining observation period. The plasma total lipid increased 6 to 7.5 times normal, reaching its highest level at the end of three months of cholesterol feeding.

Thus, the incidence of skin xanthoma increased with prolonged cholesterol feeding (Table, second line). Although hypercholesteremia must be considered a prerequisite for the development of experimental xanthomatosis, no strict correlation was observed between the degree of hypercholesteremia and the onset of skin xanthomatosis (legends, Fig. 1). The duration of cholesterol feeding proved to be an important factor in the development of xanthomatosis. In addition to plasma cholesterol concentration and "the time factor," some local tissue mechanism of unknown nature may have been responsible for lipid deposition and may explain the individual variations.

### Clinical Observations

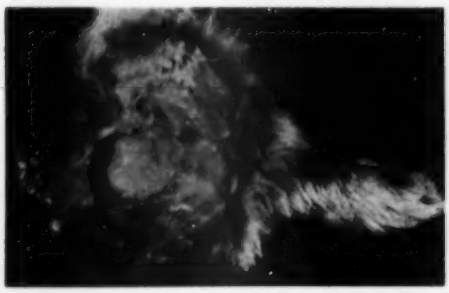
Xanthomata were noted first on the paws, as small reddish-yellow nodules, 1-3 mm. in diameter, between the toes and over the heels. The size of these nodules slowly increased to a diameter of 1-1.5 cm. (Fig. 2). The skin of the soles became thickened and cracked. In animals fed cholesterol for longer than three to six months, small elevated yellowish tophi, 1-3 mm. in diameter, were seen scattered over the ears, resembling eruptive xanthoma in man. Previous traumatic injury to the ear, e. g., at the

Fig. 2.—Rabbit 384, fed cholesterol for 160 days. Note small cutaneous xanthoma on the toe and beginning ulceration in the center (arrow).

sites of puncture for blood sampling, usually predisposed the skin to the development of xanthomatous nodules. In some instances the skin over the back and neck showed loss of hair and appeared reddish and thickened (hyperkeratosis).

In many instances, ulceration of the xanthomata and secondary infection, with abscess formation, complicated the picture. Occasionally, large sterile cholesterol abscesses were noted over the limbs. In one animal the sterile abscess assumed the size of an orange (10 cm. in diameter). In another animal two such abscesses were found, one on the upper and one on the lower limb (Fig. 3). The latter animal was fed cholesterol Chow for three months only. The sterile abscesses contained a cholesterol-rich, whitish, cheesy material cultures of which yielded no bacterial growth.

Fig. 3.—Large sterile abscess of the left forelimb of Rabbit 505 after cholesterol feeding for 95 days. Note whitish, cheesy material in the abscess.



Some of the animals fed cholesterol for more than six months showed thickening and swelling of the ankles and knee joints. There was no evidence of inflammatory changes or fluid accumulation in these joints. The firmness of the swelling suggested deposition of cholesterol in the tissues. A more careful evaluation of these changes was possible by the systematic use of roentgenological studies.

### Roentgenological Studies

In 18 animals roentgenological studies were carried out serially from the second to the eighth month of cholesterol feeding. Considerable thinning of the cortex of the long bones was noticed after three to five months. Pathological fractures occurred in three animals: In one animal fed cholesterol for two and one-half months a fracture of the lower end of the femur was observed, and in two animals fed cholesterol for five months fractures of the lower end of the tibia were seen. In animals exhibiting marked soft-tissue swelling of the paws periosteal elevation in the phalanges was usually found.

In four animals with marked swelling of the knees and/or ankles considerable thickening of both the bones and the soft tissues

Fig. 4.—Roentgenological appearance of the left hindleg of Rabbit 171 after 180 days of cholesterol feeding. Note thinning of the cortex with fan-shaped widening and osteosclerotic changes in the lower end of the tibia and in the tarsal bones, resulting in complete destruction of the ankle joint. Also notice periosteal elevation and soft-tissue swelling.

was seen roentgenologically. The lower ends of the tibiae and the fibulae were widened and presented a fan-shaped appearance, with considerable osteosclerosis and complete destruction of the ankle joints (Fig. 4).

### Atherosclerosis of the Arteries

In general, no gross atherosclerotic plaques were found after two weeks of cholesterol feeding. At the end of one month, tiny but definitely yellowish-white plaques could be demonstrated at the ascending part and the arch of the aorta, especially around the orifices of the innominate artery and the other branching arteries. Similar small plaques were noticed in the pulmonary artery, especially over the bifurcation.

With continuation of cholesterol feeding atheromatous plaques became larger, more numerous, and confluent, with longitudinal orientation. New plaques appeared at the orifices of the intercostal arteries and the branching arteries of the abdominal aorta. Thickening and beading of the mitral and aortic valves were usually present after two to three months of cholesterol feeding.[9,10]

When cholesterol feeding was carried on beyond four to six months, the atheromatous plaques of the aorta and pulmonary artery were so extensive that relatively normal intima was seen only in small skip areas.

### Microscopic Findings

Earliest skin xanthomata, not visible to the naked eye, showed foam cells, isolated or in nests in the upper corium and in perivascular and perineural spaces in the deep corium. The presence of isolated foam cells was usually accompanied by a local bluish-violet hue of the ground substance in sections stained with hematoxylin-eosin. A systematic study on the relationship between lipid deposition and alteration of the ground substance will be reported later.

A typical well-developed xanthomatous skin nodule was characterized histologically by thinning of the epidermis, marked distention of the dermis with fat-laden foam cells, and a central pool consisting of cholesterol crystals and cellular debris (Fig. 5*A* and *B*). The usual wavy contour of the epidermis became flattened. Nests of foam cells were separated by heavy strands of collagenous fibers of the dermis. No foreign-body giant cells were found.

The deposition of foam cells in the synovial membrane, periosteum, and bone marrow was noted long before the development of any roentgenological changes in the bones or joints. After two months of cholesterol feeding the synovial membranes and the periosteum showed already marked thickening due to the presence of many

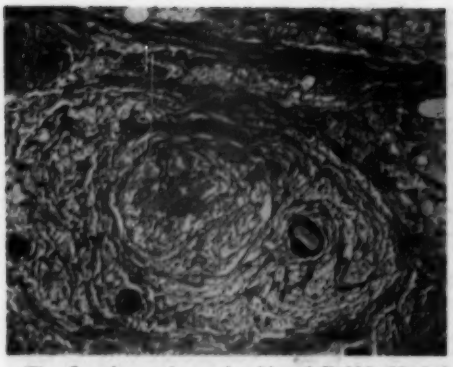
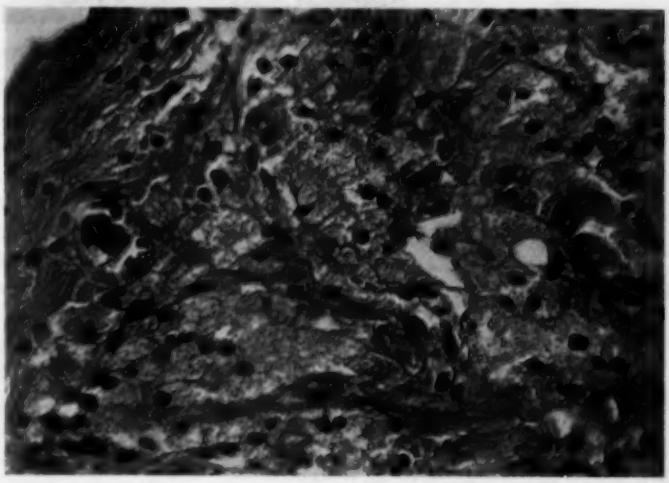


Fig. 5.—*A*, xanthoma in skin of Rabbit 82, fed cholesterol for 187 days, showing central degeneration with collection of cholesterol crystals and cellular debris. Hematoxylin-eosin stain; reduced to 57% of mag. × 70.



*B*, high-powered view of cutaneous xanthoma of the same animal. The dermis was distended with large number of foam cells. Hematoxylin-eosin stain; reduced to 63% of mag. × 800.

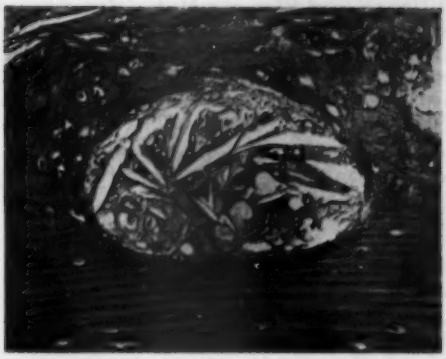
foam cells. Isolated foam cells or clusters of them were seen in the bone marrow of the long bones and of the ribs, especially in animals with active hemopoiesis. In the adult animal with inactive bone marrow of the long bones large foam cells and cholesterol crystals were very frequently observed in the bone lacunae (Figs. 6*A* and *B*, 7). In the tendons and ligaments the foam cells were usually confined to the fibrous sheath only. Occasionally, one or two isolated foam cells were seen at the insertion of a tendon or a ligament to the cartilaginous cap of a long bone. The occurrence of foam cells was thus correlated with the sites where fibroblast and undifferentiated mesenchymal cells were abundant.[13] Such tissues as cartilages, tendons, and ligaments, which are characterized by highly differentiated cells and poor blood supply, exhibited no infiltration of foam cells.

In bones with fractures the deposition of foam cells and cholesterol crystals was more abundant than in intact bones. The deposition of cholesterol was particularly heavy at the site of the fracture. Callus formation occurred in tissues loaded with cholesterol crystals and large numbers of foam cells. It seemed as though the heavy deposition of cholesterol crystals precipitated pathological fracture of the bone. On the other hand, it has been well known that any injury to the tissues invited the deposition of cholesterol. It would be interesting to investigate the healing process in normal and cholesterol-fed animals.

## Summary

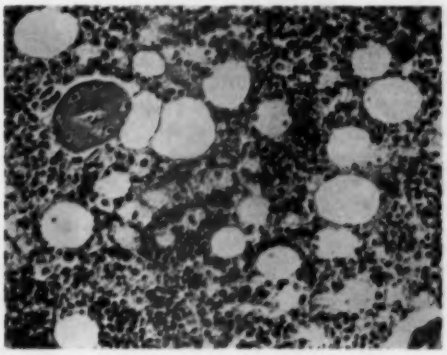
The occurrence and the clinical and roentgenological appearance of experimental skin, joint, and bone xanthomatosis in cholesterol-fed rabbits are described. The changes in levels of plasma cholesterol, phospholipids, and total lipids are presented, and the gross appearance of aortic atherosclerosis in these animals is briefly men-

Fig. 6.—*A*, deposition of foam cells in synovial membrane in the joint of Rabbit 458, killed after 270 days of cholesterol feeding. Hematoxylin-eosin stain; reduced to 63% of mag. × 800.



*B*, clusters of cholesterol crystals in Howship's lacuna of the femoral cortex of the same animal as that in *A*. Hematoxylin-eosin stain; reduced to 57% of mag. × 400.

Fig. 7.—Bone marrow showing the presence of large number of foam cells. Rabbit 379, fed cholesterol for 105 days. Hematoxylin-eosin stain; reduced to 57% of mag. × 400.



tioned. Microscopic xanthomatosis of the skin and joints is evident as foam cells accumulate in the corium of the skin, in the synovial membranes, in the tendon sheaths, in the bone marrow, and in Howship's lacunae of the bones. The infiltration of foam cells seems to occur in tissues characterized by an adequate blood supply, abundance of fibroblasts, and undifferentiated mesenchymal cells.

Mount Sinai Hospital.

## REFERENCES

1. Anitschkow, N.: Experimental Arteriosclerosis in Animals: A Survey of the Problem, edited by E. V. Cowdry, New York, The Macmillan Company, 1933, Chap. 10.

2. Rusch, H. P.; Bauman, C. A., and Kline, B. E.: Production of Xanthoma in Rabbits, Arch. Path. 28:163, 1939.

3. Kuntz, A., and Sulkin, N. M.: Lesions Induced in Rabbits by Cholesterol Feeding with Special Reference to the Origin, Arch. Path. 47: 248, 1949.

4. Wang, C. I.; Schaefer, L. E.; Drachman, S. R., and Adlersberg, D.: Plasma Lipid Partition of the Normal and Cholesterol-Fed Rabbit, J. Mt. Sinai Hosp. 21:19, 1954.

5. Adlersberg, D.: Inborn Errors of Lipid Metabolism: Clinical, Genetic, and Chemical Aspects, A. M. A. Arch. Path. 60:481, 1955.

6. Sperry, W. M., and Webb, M.: A Revision of the Schoenheimer-Sperry Method for Cholesterol Determination, J. Biol. Chem. 187:97, 1950.

7. Sperry, W. M.: Electrophotometric Microdetermination of Phosphorus in Lipid Extracts, Indust. & Engin. Chem., Anal. Ed. 14:88, 1942.

8. Bloor, W. R.: Studies on Blood Fat: Variations in the Fat Content of the Blood Under Approximately Normal Conditions, Biochem. J. 19:1, 1914.

9. Wang, C. I.; Schaefer, L. E., and Adlersberg, D.: Tissue Permeability—A Factor in Atherogenesis, Circulation Res. 3:293, 1955.

10. Wang, C. I.; Schaefer, L. E., and Adlersberg, D.: Experimental Studies on the Relations Between Adrenal Cortex, Plasma Lipids and Atherosclerosis, Endocrinology, 56:628, 1955.

11. Ham, H.: Histology, Ed. 2, Philadelphia, J. B. Lippincott Company, 1953, p. 840.

# News and Comment

## PERSONAL NEWS

**Dr. Herwig Hamperl Visiting Professor at University of Wisconsin Medical School.**—Dr. Herwig Hamperl, director of the Institute of Pathology, University of Bonn, Germany, has been appointed visiting Carl Schurz Professor at the University of Wisconsin Medical School for the spring semester. Dr. Hamperl will present lectures at the University Medical School and at the University of Wisconsin, Milwaukee Extension Division.

**Colonel Blumberg Appointed Army Deputy Director, Institute of Pathology.**—Col. Joe Morris Blumberg has been appointed Army Deputy Director of the Armed Forces Institute of Pathology. He succeeds Col. Francis E. Council, who retired from the Army on March 31, 1957.

**Dr. William W. Hurteau Appointed to Methodist Hospital, Indianapolis.**—Dr. Hurteau has joined the Department of Clinical Laboratories, Methodist Hospital, Indianapolis.

**Appointment of Dr. Alexander Symeonidis to Cancer Institute, Aristotelian University School of Medicine.**—Dr. Symeonidis has been made professor of pathology and director of the Cancer Institute of the Aristotelian University School of Medicine, Thessaloniki, Greece. He was formerly head of the geographic pathology unit of the National Cancer Institute.

**Dr. Harry S. N. Greene Given Borden Award.**—Dr. Harry S. N. Greene, Anthony N. Brady professor of pathology at Yale University School of Medicine, has been given the 1956 Borden Award in the Medical Sciences. This award, consisting of a gold medal and $1000, was given in recognition of his basic researches in the field of oncology.

## ANNOUNCEMENTS

**The Adam M. Miller Memorial Lecture.**—The 21st Adam M. Miller Memorial Lecture of the State University of New York College of Medicine was given on Dec. 19 by Dr. G. Kenneth Mallory, of Boston. Dr. Mallory spoke on the subject "The Development, Healing, and Complications of Myocardial Infarction."

The 12th annual Hanau W. Loeb Lectureship of the St. Louis University School of Medicine was given by Dr. Shields Warren, of Boston. He discussed "Man and His Atomic Environment."

## SOCIETY NEWS

**American Goiter Association.**—The American Goiter Association will hold its annual meeting at the Hotel Statler, New York, May 28, 29, and 30, 1957.

## GENERAL NEWS

**Responsibilities of the Medical Profession in the Use of X-Rays and Other Ionizing Radiation** (Statement by the United Nations Scientific Committee on the Effects of Atomic Radiation).—1. The United Nations General Assembly, being aware of the problems in public health that are created by the development of atomic energy, established a Scientific Committee on the Effects of Atomic Radiation. This Committee has considered that one of its most urgent tasks was to collect as much information as possible on the amount of radiation to which man is exposed today, and on the effects of this radiation. Since it has become evident that radiation due to diagnostic radiology and to radiotherapy constitutes a substantial proportion of the total radiation received by the human race, the Committee considers it desirable to draw attention to information that has been obtained on this subject.

2. Modern medicine has contributed to the control of many diseases and has substantially prolonged the span of human life. These results have depended in part on the use of radiation in the detection, diagnosis, and treatment of disease. There are, however, few examples of scientific progress that are not attended by some disadvantages, however slight. It is desirable therefore to review objectively the possible present or future consequences of increased irradiation of populations which result from these medical applications of radiation.

3. It is now accepted that the irradiation of human beings, and particularly of their germinal tissues, has certain undesirable effects. While many of the somatic effects of radiation may be reversible, germinal irradiation normally has an irreversible and therefore cumulative effect. Any irradiation of the germinal tissues, however slight, thus involves genetic damage which may be small but is nevertheless real. For somatic effects there may, however, be thresholds for any irreversible effects, although if so these thresholds may well be low.

4. The information so far available indicates that the human race is subjected to natural radiation, as well as to artificial radiation due to its medical applications, to atomic industry and its effluents, and to the radioactive fall-out from nuclear explosions. The Committee is aware of the potential hazards that such radiation involves, and it is collecting and examining information on these subjects. [The radiation due to natural sources has been estimated to produce between 70 and 170 mrem of radiation to the gonads per annum in most parts of certain countries in which it has been studied, although higher values are found locally in some areas—from the reports "Hazards to Man of Nuclear and Allied Radiations," published by the United Kingdom Medical Research Council in June, 1956, in which also the millirem is defined; and from information submitted to the Committee.]

5. The amount of radiation received by the population for medical purposes is now, in certain countries, the main source of artificial radiation and is probably about equal to that from all natural sources. Moreover, since it is given on medical advice, the medical profession exercises responsibility in its use.

6. The Committee appreciates fully the importance and value of the correct medical use of radiation, in the diagnosis of a large number of conditions, in the treatment of many such diseases as cancer, in the early mass detection of conditions such as pulmonary tuberculosis, and in the extension of medical knowledge.

7. Moreover, it appreciates fully the contribution of the radiological profession, through the International Commission on Radiological Protection [see the report of the Commission, *Brit. J. Radiol.* 27:245 (April) 1954; Supp. 6, Dec., 1954; *J. franç. electro-radiol.* Oct., 1955] in recommending maximum permissible levels of irradiation. As regards those whose occupation exposes them to radiation, the establishment of these levels depends on the view that there are doses which, according to present knowledge, do not cause any appreciable body injury in the irradiated individual; and also on the consideration that the number of people concerned is sufficiently small for the genetic repercussions upon the population as a whole to be slight. Whenever exposure of the whole population is involved, however, it is considered prudent to limit the dose of radiation received by germinal tissue from all artificial sources to an amount of the order of that received from the natural background radiation.

8. It appears most important therefore that medical irradiations of any form should be restricted to those which are of value and importance, either in investigation or in treatment,

so that the irradiation of the population may be minimized without any impairment of the efficient medical use of radiation.

9. The Committee is consequently anxious to receive information through appropriate governmental channels as to the methods and the extent by which such economy in the medical use of radiation can be achieved, both by avoiding examinations which are not clearly indicated and by decreasing the exposure to radiation during examinations, particularly if the gonads, or the fetus during pregnancy, lies in the direct beam of radiation. It seeks, in particular, to obtain information as to the reduction in radiation of the population which might be achieved by improvements in instrument design by fuller training of personnel, by local shielding of the gonads, by choosing appropriately between radiography and fluoroscopy, and by better administrative arrangements to avoid any necessary repetition of identical examinations.

10. The Committee also seeks the cooperation of the medical profession to make possible an estimate of the total radiation received by the germinal tissue of the population before and during the childbearing age. It considers it to be essential that standardized methods of measurement, of types at present available, should be widely used to obtain this information, and it emphasizes the value of adequate records, maintained by those using radiation medically, by the dental profession, and by the responsible organizations in allowing such radiation exposure to be evaluated. The Committee is convinced that information of this type will make it possible to decrease the total medical irradiation of the population while preserving and increasing the true value of the medical uses of radiation.

**Sixth Conference of International Society of Geographic Pathology.**—The Sixth Conference of the International Society of Geographic Pathology will be held in Paris, July 9-12, 1957. Individual communications on subjects regarding gastroduodenal ulcers or papers dealing with general problems of geographic pathology will be welcome at the meetings. One half-day will be devoted to these. The papers are limited to 10 minutes, and the author should send the title, name, address, and a short summary of approximately 10 lines to the general secretary, Prof. Fred C. Roulet, Basel, Switzerland, St. Albanrheinweg 174.

# Books

## BOOK REVIEWS

**Medical Parasitology.** Second Edition. By William G. Sawitz. Price, $6.00. Pp. 342, with 80 illustrations. McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1956.

This second edition includes significant changes only in the chapter on treatment, which has been brought up to date, particularly as to the use of oxytetracycline. A brief synopsis has been added which includes some useful tables but is perhaps redundant, since the entire book is essentially a synopsis of medical parasitology. In this age of expert biological photography, a more useful addition would have been photographic plates to accompany the drawings. These illustrations are essentially unchanged. More attention has been given to dimensional scales. The oocysts of Isospora hominis appear to have shrunken since the first edition.

The work remains an important and practical contribution to the education of the busy medical student and physician.

**Clinical Examination in Neurology.** By members of Section of Neurology and Section of Physiology, Mayo Clinic and Mayo Foundation for Medical Education and Research, Graduate School, University of Minnesota, Rochester, Minn. Price, $7.50. Pp. 370, with 76 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1956.

This compendium by sixteen Mayo Clinic members is intended as a practical guide to the performance of the clinical neurologic "work-up." Much of the material is particularly directed to the Mayo Clinic trainee in neurology. It includes brief discussions and useful illustrations of underlying anatomic and physiologic principles. There are chapters on neuro-ophthalmology, mental function, language and motor speech, electroencephalography, and electromyography. A brief (twenty pages) chapter on biochemical and pharmacological aids in neurologic diagnosis is up-to-date, but not sufficiently detailed for practical laboratory use. There is no bibliography.

**An Atlas of Otolaryngic Pathology.** Col. J. E. Ash (M.C.) (Ret.), U. S. Army, and Muriel Raum, M.D. Price, $20.00. Pp. xii + 572, with 2024 figures on 420 plates (2 color). Armed Forces Institute of Pathology, American Registry of Pathology, 14th St. and Alaska Ave. N. W., Washington, D. C., 1956.

This volume is as much a tribute to medical photography as it is to otolaryngic pathology. The illustrations of clinical, gross, and microscopic pathology are equally superb. Dr. Raum is to be congratulated for her capable and comprehensive coverage of this illustrative material. The text, written by Colonel Ash, is comparatively succinct. Included is a series of twelve chapters on pertinent general pathology, designed for the clinical otolaryngologist. The bibliography is brief, but intelligently chosen. While this "Atlas" is easily the best of its kind currently available, it is perhaps less useful to the surgical pathologist than its earlier-appearing sister volume on opthalmic pathology.

**The Prevention of Disease in Everyday Practice.** Isadore Givner and Maurice Brugner Editors, with 47 contributors. Price, $20.00. Pp. 964, with 50 illustrations. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1955.

The prevention of disease in the individual patient should be of the utmost importance to the practitioner of medicine, and a volume such as this is long overdue. Most of the specialized fields of internal medicine, surgery, pediatrics and gynecology, psychiatry, radiology, ophthalmology, otolaryngology, etc., are treated, often by distinguished authors in the various fields. The prevention both of primary disease and of the complications of disease is treated, the latter perhaps too extensively, since these topics are well covered in the usual textbooks of the various fields. This volume should be useful to those in the general practice of medicine. The specialist, too, may find useful and important information in the sections devoted to his particular practice.

**Neoplasms of the Domesticated Mammals (A Review).** By E. Cotchin, M.R.C.V.S. Price, about $3.00. Pp. 100. Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, 1956.

This small book reviews briefly the benign and malignant tumors of horses, cattle, sheep, pigs, dogs, cats, and a few other species. The excellent bibliography is large, but there are no illustrations. A chapter is devoted to each of the major tissues and organ systems. A wide variety of tumors in animals, great species variations in frequency and site, and interesting differences from the human tumor spectrum are apparent. This book should be interesting and useful to many people.

PAUL E. STEINER, M.D.

---

# Correspondence

## Esterase Activity of Developing Bones and Teeth

*To the Editor*: In my article "Esterase Activity of Developing Bones and Teeth," in the February issue of the ARCHIVES (p. 164) the following material should be added to the data presented in the right-hand column on the first page of the article:

(*f*) Naphthol AS-LT acetate

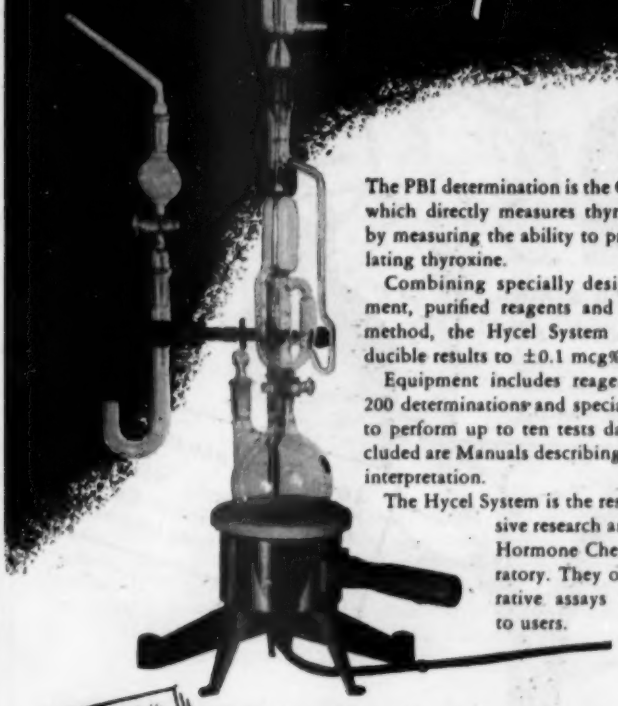
Analysis: calcd. C, 72.19%, H 5.75%, N 4.01%
found C 71.95%, H 5.94%, N 4.2%
m. p. 163 C.

This substrate was found to be rapidly hydrolized by developing bone.

M. S. BURSTONE, Bethesda, Md.

## FOR PROTEIN BOUND IODINE DETERMINATIONS

# ...The Hycel System

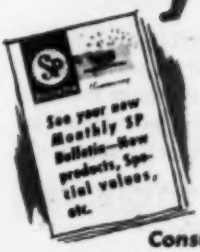


The PBI determination is the ONLY TEST which directly measures thyroid function by measuring the ability to produce circulating thyroxine.

Combining specially designed equipment, purified reagents and a simplified method, the Hycel System gives reproducible results to ±0.1 mcg%.

Equipment includes reagents for over 200 determinations and special equipment to perform up to ten tests daily. Also included are Manuals describing method and interpretation.

The Hycel System is the result of extensive research and testing by Hormone Chemistry Laboratory. They offer corroborative assays as a service to users.




**No. 53845—Hycel PBI System.....$340.00**

A multiple set is available containing sufficient reagents for over 600 tests, and additional equipment for performing 20 or more tests daily. Our catalog No. 53846, it sells for $1050.00.

***Consult your SP representative for further details.***

**You can rely on Scientific Products—**

Ainsworth balances
American Optical microscopes
B.B.L. culture media
Barnstead stills
Bausch & Lomb microscopes
Beck Lee E.K.G. equipment
Beckman instruments
Becton-Dickinson equipment
Berkeley radioisotope equipment
Browne-Morse sectional cabinetry
Christian Becker balances
Clay Adams medical equipment
Coleman spectrophotometers
Coors porcelain
Corning glass
Dade serums
Di3Pa Home—disposable specialties
Harleco-Loddon chemicals
Hycel clinical testing equipment
International centrifuges
Jewett refrigerators
Kimble glassware
Labline laboratory equipment
Lindberg hotplates & furnaces
Lipshaw tissue equipment
Mallinckrodt reagent chemicals
Matheson Coleman & Bell organic chemicals
Monaghan respiratory equipment
Ohaus scales
Precision scientific equipment
Schieffelin pharmaceuticals
SP laboratory specialties
Spinco electrophoresis apparatus
Sylvana serological reagents
Torsion balances
Virtis virus & tissue equipment
Warner-Chilcott pharmaceuticals

**No matter what your laboratory needs—be sure to consult your SP representative first!**

# Scientific Products

DIVISION OF AMERICAN HOSPITAL SUPPLY CORPORATION

New York • Chicago • Kansas City • Minneapolis • Atlanta • Washington • Dallas • Los Angeles • San Francisco

# fine "family" to get acquainted with...

technicon DEHYDRANT
non-hardening: non-distorting: non-hygroscopic: non-volatile: lasts longer
technicon FIXATIVE
penetrates rapidly: completely stable: long-lived: precipitate-free
technicon CLEARANT
non-hardening: non-volatile: non-hygroscopic: clears fat: lasts longer
technicon STAINS
highly selective: standardized characteristics: chemically balanced: never needs filtering
technicon MOUNTING MEDIUM
dries quickly: chemically inert: won't crystallize or darken: higher refractive index
technicon PARAWAY
paraffin solvent: won't "dry out": shrink or distort tissues: lasts longer

**These are the members of the Technicon family of standardized histologic reagents. Many pathologists consider them the closest approach yet made to ideal media for routine laboratory use.**

**Not least among their outstanding characteristics is their common high flash point which makes them mandatory for use with Autotechnicon for Underwriter's Laboratory approval.**

*For details and specimen methods, let us send you our Bulletin R-2 "Standardized Histologic Reagents."*

**technicon®**

**standardized histologic reagents**

**TECHNICON CHEMICAL COMPANY, INC.**

Chauncey, New York

Paris Office: COMPAGNIE TECHNICON • 7 rue Georges Villa • Paris

**PARAGON STAINS** **PARAMOUNT QUALITY**

# PARAGON STAINING SOLUTIONS

**For Tissue Sections**

Dependable—Today; Tomorrow; Every Day

With Paragon Staining Solutions you obtain superbly stained tissue sections. The brilliance and sharpness of stain without diffusion or unpredictable characteristics greatly facilitates diagnosis.

HEMATOXYLIN STAIN—PARAGON (aqueous alum hematoxylin). Made from our own formula. Yields vivid, sharply stained blue nuclei that are really blue—not off color or muddy. Extremely sharp staining and selective with no diffusion. Full bodied and strong. For a given staining time, repeatedly duplicates depth of staining from slide to slide—every day.

| | | |
|---|---|---|
| PS1101 | Bottle (500 cc) | $2.25 |

EOSIN STAIN—PARAGON (alcoholic). A special eosin compound of our own preparation. Produces deep brilliant red counterstains. Packed in two forms—ready to use and concentrated (requiring the addition of 3 parts of 95% alcohol).

| | | |
|---|---|---|
| PS1201D | Bottle (500 cc) ready to use | $2.25 |
| PS1201 | Bottle (250 cc) for 1000 cc | 3.00 |

ELASTIC FIBER STAIN—PARAGON. Our own resorcin-fuchsin modification of Weigert's Elastic Fiber Stain. Relieves the laboratory of the laborious work involved in the preparation of this important stain. Stains sharply with no diffusion into other tissue components.

| | | |
|---|---|---|
| PS1225 | Bottle (250 cc) | $2.65 |

VAN GIESON STAIN—PARAGON. Especially designed to produce brilliant differential counterstaining with less tendency to wash out in rinsing alcohols.

| | | |
|---|---|---|
| PS1250 | Bottle (250 cc) | $1.50 |

PARAGON MULTIPLE STAIN FOR FROZEN SECTIONS. Invaluable to the Pathologist where seconds count and the Surgeon waits for the diagnosis. A single solution which stains instantaneously yielding a hematoxylin-eosin like picture. No special technic. With Paragon Mounting Medium For Frozen Sections (water soluble) section is stained, mounted and under microscope in less than one minute.

| | | | |
|---|---|---|---|
| PS1301 | Paragon Multiple Stain For Frozen Section | Bottle (50 cc) | $2.00 |
| P451 | Paragon Mounting Medium For Frozen Sections | Bottle (25 cc) | .50 |

Request samples on your institution letterhead.
Write for fully descriptive catalog number 1049 A which includes a descriptive section on staining technics.

All prices F. O. B. New York, New York, subject to change without notice

**Manufactured exclusively by**

**PARAGON C. & C. CO., Inc. - 2540 Belmont Ave, New York 58, N. Y.**

**Cable Address: Wijeno, New York**

---

Write for details on the following Paragon Staining Solutions:

ACID FAST BACTERIA STAIN • CRYSTAL VIOLET STAIN • GRAM'S IODINE SOLUTION
SAFRANIN STAIN • LOEFFLER'S ALKALINE METHYLENE BLUE • ZIEHL-NEELSEN STAIN • WRIGHT'S STAIN • BUFFER SOLUTION FOR WRIGHT'S STAIN

## *PATHOLOGIST*

**To serve in the newly reorganized modern laboratories of the Medical Examiner's Office of the City of Philadelphia. Duties include supervision of work performed by subordinate pathologists. Prior medico-legal experience not required. Starting salary $13,142 per year. 37½ hour work-week. University or hospital affiliations are encouraged. Qualifications for appointment are eligibility for certification in pathologic anatomy, clinical pathology or both, or recognized sub-specialty, issued by the American Board of Pathology.**

***Additional information and an application form may be obtained by communicating with Joseph W. Spelman, M.D., Medical Examiner, 13th and Wood Streets, Philadelphia 7, Pennsylvania.***

Here are several booklets on mental hygiene that may prove of interest and use to you and your patients.

**EMOTIONAL HEALTH IN WORK AND PLAY**
T. R. Retlaw, 8 pages, 15 cents

**HYPNOTISM—HUMBUG OR HEALING**
James A. Brussel, 6 pages, 10 cents

**YOUR STAKE IN MENTAL HEALTH**
George S. Stevenson, 4 pages, 10 cents

**THE PSYCHIATRIST** Edward Dengrove and Doris Kulman, 6 pages, 10 cents

## BOOKLETS ON MENTAL HYGIENE

Keep a supply of these booklets on hand. Available at lower cost for quantity purchases.

**AMERICAN MEDICAL ASSOCIATION**
535 North Dearborn Street • Chicago 10 • Illinois

## A. M. A. AMERICAN JOURNAL OF DISEASES OF CHILDREN

*integrate your knowledge—subscribe to . .*

↓

A. M. A. Archives of PATHOLOGY

↓

A. M. A. Archives of INTERNAL MEDICINE

↓

A. M. A. Archives of OPHTHALMOLOGY

↓

A. M. A. Archives of OTOLARYNGOLOGY

For a more complete reference shelf, we recommend these related journals. The variety of fields covered are especially appealing to the general practitioner with special interests. Each issue is important to your practice. Begin your subscriptions TODAY.

Department (D. C.)
AMERICAN MEDICAL ASSOCIATION
535 N. Dearborn St.
Chicago 10, Ill.

Begin my subscription(s) NOW, for:

A. M. A. .....

NAME .....

STREET .....

CITY .....STATE .....

# Thanks to radiography and photography

## *physicians and students can review this classic case...*

1. RESECTION OF COARCTATION OF AORTA: Radiograph shows enlarged heart and eroded ribs—indications of the condition.

2. Resected coarctate segment of the aorta.
*Turn page for color illustrations of surgery.*

With the visual data reproduced on these pages—preoperative radiograph of the patient's condition, color photographic record of the surgical procedure—the attending physician, the radiologist and the surgeon have material for discussion, for teaching and research, for publication and exhibition. Like ripples from a pebble thrown in the water, experience becomes an ever-widening force!

Kodak

Anytime — anywhere — physicians, students, researchers who have access to the material shown here, can study this case, view the radiographs, review the steps in surgery, learn from the problem and its solution.

The cost of this visual data? Small in comparison to its great educational value!

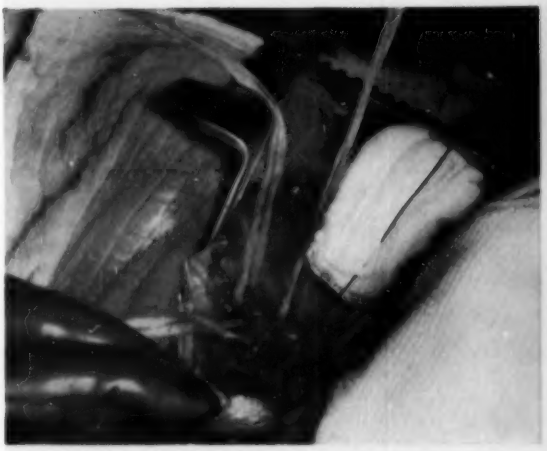
**For Radiography:** Kodak Blue Brand X-ray Film and Kodak x-ray processing chemicals meet the most exacting requirements. They are always dependable—uniform. Quality-controlled—rigidly tested—they are made to work together.

**For Color Photography:** Kodachrome Films for miniature and motion-picture cameras; Kodak Ektachrome Films and Kodak Ektacolor Films for sheet-film cameras; Kodak Ektachrome Films for roll-film and miniature cameras; Kodacolor Films for roll-film cameras and cameras accepting No. 828 film. Kodak color print materials are also available.

*Order x-ray products*
*from your x-ray dealer, photographic products*
*from your photographic dealer.*

EASTMAN KODAK COMPANY
*Medical Division*
Rochester 4, N. Y.

Kodak
TRADE MARK



3. Exposure of coarctation and *ligamentum arteriosum*.

4. Coarctation of aorta with distal dilatation and large intercostal arteries.

5. Completion of end-to-end anastomosis of aorta following resection of the coarctation.

## How to clear one of your laboratory shelves...

We don't really expect you to throw away your stock solutions, but... Versatol in whole human serum can now be used as a *standard* for twelve important blood chemistry tests. Versatol takes the place of individual aqueous standards whenever a standard is called for in your procedure – in making calibration curves, in titrating unknowns, in flame photometric methods. Because Versatol duplicates the interfering substances in blood, errors actually *caused* by aqueous solutions are eliminated.

And Versatol is also used as a *control* serum to check equipment, reagents, and technique. Here, too, Versatol gives the most accurate results, since each determination is performed in the presence of all the complex substances in the patient's blood. Versatol contains known normal amounts of serum protein-bound iodine . . . total nitrogen . . . total protein . . . nonprotein nitrogen . . . urea nitrogen . . . creatinine . . . sodium . . . potassium . . . chloride . . . phosphate . . . calcium . . . glucose.

Versatol eliminates the tedious time-consuming task of preparing many different aqueous standards. It is prepared simply by adding distilled water.

Available in boxes of ten 5-ml. vials, $21.00, from leading laboratory supply distributors.

**Versatol**
TRADEMARK

*Laboratory Supply Division*
**WARNER-CHILCOTT**
MORRIS PLAINS, N. J.

E

Please send me

- ☐ More information on Versatol
- ☐ Part I of Warner-Chilcott Blood Chemistry Manual

Please ship through my nearest distributor _____ boxes of Versatol ☐

NAME_____

LABORATORY_____

ADDRESS_____

CITY_____ STATE_____

**the new Autotechnicon® takes a third less space has 20% more capacity**

actual size on 10 foot bench

**From the very beginning . . .** in the very first Autotechnicon which introduced automation to tissue-processing . . . a round deck was used because only in a circle can you condense so many beakers, so compactly. It's simple geometry.

But even the best idea can be improved, the deck is still a perfect circle . . . but it is smaller by a third. Still, the new radial beakers have 20% more capacity of both fluid and tissues than the previous ones.

Since it doesn't sprawl all over the place, as you see above, the New Autotechnicon, occupies only the very corner of your lab-bench, leaving almost the entire bench free and clear for other work.

the entire Autotechnicon is mounted on a free-wheeling quiet turntable . . . just rotate it to bring any beaker to the front

*Write today for Booklet 2-AT, for description of these new instruments.*

*the* Autotechnicon®

Trailblazer in histologic automation

**THE TECHNICON COMPANY**

Chauncey New York

Paris Office: COMPAGNIE TECHNICON • 7 rue Georges Ville • Paris